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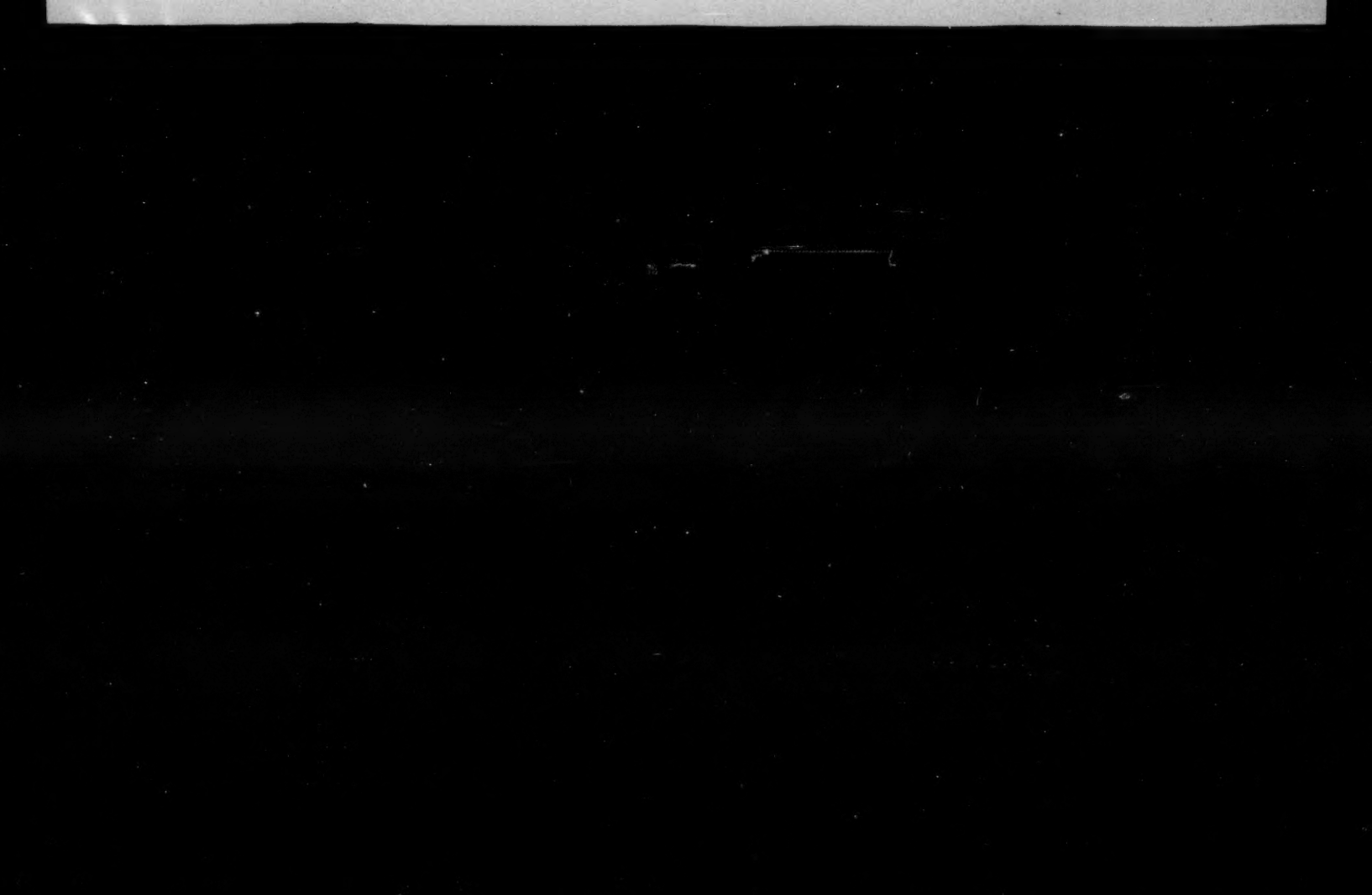

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THE INFLUENCE OF LIMITED FEEDING, USING HIGH FIBER RATIONS, UPON GROWTH AND CARCASS CHARACTERISTICS OF SWINE.[1] I. EFFECTS UPON FEED-LOT PERFORMANCE

R. A. MERKEL,[2] R. W. BRAY, R. H. GRUMMER, P. H. PHILLIPS AND G. BOHSTEDT

University of Wisconsin[3]

SWINE are unique in that they utilize concentrate feed much more efficiently than other meat animals and they readily convert feed into fat, which is deposited in the numerous depots of the body. Self-feeding high energy rations intensifies the deposition of fat. Thus, feeding for economical production of high quality "lean pork" presents a problem to swine producers, particularly since consumers prefer pork with a moderate amount of fat. In addition competition with other shortenings has reduced the market value of lard, thereby decreasing the value of overfat hogs.

McMeekan and Hammond (1939) and McMeekan (1940), found that a high level of nutrition during the early stages of growth followed by a low level of nutrition during the later stages of growth of swine, intensifies early-developing tissues (skeletal framework and muscle) and inhibits later-developing tissue (sub-cutaneous fat), thus producing a lean hog. The opposite effect is produced when the nutritional levels are reversed. Since the plane of nutrition exhibits on influence upon the physical development of animals, it follows logically that attempts to alter carcass composition might well begin with a practical and profitable feeding regime which will favor muscular development and retard fat deposition.

Restricting feed intake of swine by hand limited-feeding produces pork carcasses with less fat as reported by Ellis and Zeller (1931–32), (1934), Shorrock (1940), Winters *et al.* (1949), and Brugman (1950). However, labor and cost of hand limited-feeding are prohibitive for practical use. Results comparable to restricted feeding were observed by Axelsson and Eriksson (1953) when the level of crude fiber in the dry matter content of the ration was increased, even though the pigs were allowed to eat ad libitum. Limiting the digestible nutrients of the ration by adding high fiber or relatively indigestible ingredients is adaptable to self-feeding and has been employed in several recent studies, Whatley

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et al. (1951), Coey and Robinson (1954), Crampton *et al.* (1954), and Bohman *et al.* (1955).

The data in this and the succeeding report were obtained from hogs self-fed low energy rations in an attempt to improve carcass acceptability.

Experimental

This experiment was designed to limit-feed in dry lot by restricting the level of TDN in the ration with fibrous feeds. A basal ration consisting of 18% protein and 76% TDN (from weaning to 125 lb.) and 14% protein and 78% TDN (from 125 lb. to slaughter wt. at 210 lb.) was diluted with two levels of corn cobs and two levels of "poor quality" alfalfa hay to arrive at low (62) and medium (69) TDN levels. The alfalfa hay contained a high proportion of stem to leaves and was extremely weathered. The corn cobs and alfalfa hay were ground to pass a ⅛ inch screen and mixed with the other ingredients of the ration. Lot 2 was hand-fed the basal ration twice daily to restrict feed consumption to a level approximately 70% of that consumed by the hogs self-fed the basal ration, lot 1. The remaining lots were self-fed. The rations in lots 3 and 4 were similiar to the basal ration with the same ingredients and level of TDN, but contained 22% and 18%, and 14% and 11% protein respectively, for the first and second feed periods. High and low protein levels were included to serve as controls for the level of protein which varied inversely with the level of TDN in the rations containing corn cobs or alfalfa hay. Lots 5 and 6 contained the basal ration diluted with corn cobs to provide medium and low TDN levels. Lots 7 and 8 likewise contained medium and low TDN levels, but the basal ration was diluted with "poor quality" alfalfa hay. Composition of the rations appears in table 1. When necessary rations were supplemented with calcium and phosphorus (steamed bone meal) to meet NRC recommendations and all rations were fortified with 90 IU of vitamin D per pound of feed (irradiated yeast). Trace mineralized salt and Aurofac were added to all the rations at the rate of ½% and 1% respectively. The Aurofac provided 36 gm. of chlortetracycline and 36 mg. of vitamin B_{12} per ton of feed. Water was furnished ad libitum at all times.

Sixty-four weanling pigs of mixed breeding were equally divided according to sex and 4 pigs of each sex were randomly assigned to each of the 8 lots. Individual live weights and feed consumption for each lot were obtained at 2-week intervals. The pigs were individually removed from the experiment as they reached 210 lb. live weight, held off feed for 12 to 18 hours and slaughtered in the University meat laboratory.

The pigs were slaughtered and dressed packer style except the hams were not faced. The carcasses were split with head on and the measurement from snout to the hind foot (anterior edge of the toe) was taken on the right side of each carcass. The head was then removed and the carcasses were chilled for 24 to 48 hours at 32–34° F. Dressing percentages

were calculated from the chilled carcass weight obtained prior to cutting. Fatback thicknesses were obtained on the right side of the carcass, at the following three reference points: first rib, last rib, and last lumbar vertebra. The carcasses were separated into wholesale cuts and trimmed in

TABLE 1. COMPOSITION OF THE RATIONS

	Lot 1 Basal 76% TDN	Lot 2 Hand-fed 70% of basal	Lot 3 High protein 76% TDN	Lot 4 Low protein 76% TDN	Lot 5 Corn cobs 69% TDN	Lot 6 Corn cobs 62% TDN	Lot 7 Alf. hay 69% TDN	Lot 8 Alf. hay 62% TDN
Ingredients, %								
Oats								
1st period [a]	14.70	14.70	14.70	14.70	12.35	10.09	10.64	6.67
2nd period [a]	.....	.....	.....	.....	.....	.....	.....	.....
Tankage								
1st period	4.90	4.90	4.90	4.90	4.12	3.40	3.55	2.26
2nd period	1.95	1.95	1.96	1.95	1.66	1.36	1.37	0.88
Afalfa meal								
1st period	4.90	4.90	4.90	4.90	4.12	3.40	3.55	2.26
2nd period	4.88	4.88	4.90	4.88	4.09	3.41	3.53	2.35
Corn								
1st period	56.86	56.86	46.07	68.13	47.73	38.99	41.16	25.60
2nd period	79.98	79.98	69.59	88.76	65.55	51.67	59.99	41.96
SBOM								
1st period	16.66	16.66	27.45	5.39	14.02	11.35	12.02	7.55
2nd period	10.73	10.73	21.57	1.95	10.63	10.70	5.68	0.88
Corn cobs								
1st period	.....	.....	.....	.....	15.68	30.08	.....	.....
2nd period	.....	.....	.....	.....	15.61	30.16	.....	.....
Afalfa hay								
1st period	.....	.....	.....	.....	.....	.....	27.58	53.92
2nd period	.....	.....	.....	.....	.....	.....	27.45	51.95
Aurofac								
1st period	0.98	0.98	0.98	0.98	0.98	0.97	0.99	0.98
2nd period	0.97	0.97	0.98	0.97	0.97	0.97	0.98	0.98
Bone meal								
1st period	0.49	0.49	0.49	0.49	0.49	0.98	.....	.....
2nd period	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49
Trace min. salt								
1st period	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49
2nd period	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49
TDN, %: [b]								
1st period	76.40	76.40	76.21	76.59	69.03	62.14	68.97	61.77
2nd period	78.51	78.51	78.35	78.67	70.78	63.56	70.50	63.36
Fiber, %: [b]								
1st period	5.24	5.24	5.65	4.81	9.53	13.56	12.67	19.85
2nd period	3.14	3.14	3.52	2.80	7.83	12.24	11.07	18.17
Protein, %: [b]								
1st period	18.15	18.15	22.07	14.05	15.63	13.23	16.85	15.59
2nd period	14.09	14.09	18.12	10.99	12.90	11.73	13.22	12.33

[a] Av. wt. of pigs: 1st period, 36–125 lb.; 2nd period, 125–210 lb.
[b] These percentages are calculated from Morrison's values (1950).

accordance with the standards established by the Pork Evaluation Committee of the Fifth Annual Reciprocal Meat Conference (1952). In addition, the standards described for measuring carcass length, length of hind leg and length of ham were employed. Length of neck was measured from the atlas joint to the anterior edge of the first rib.

Lean cuts were expressed as percentages of the chilled carcass. Percentage ham represents the skinned ham and percentage ham muscle is the completely skinned, defatted and boned ham. Viscera and liver were expressed as percentages of live weight at slaughter.

Three cores of fat were removed from the center, anterior and posterior ends of the fatback with a 15 mm. cork borer and comminuted for the Hanus Iodine number determination, A.O.A.C. (1950). The areas of the ham butt and longissimus dorsi muscle (at the last rib) were obtained from tracings with a compensating polar planimeter.

These data were subjected to Snedecor's (1946) analysis of variance. The growth data and carcass characteristics each were correlated with TDN, fiber and protein level.

Results and Discussion

The composition of the rations as indicated in table 1 shows that approximately 15.5% and 30% corn cobs were added to the basal ration to arrive at the medium and low levels of TDN; whereas, approximately 27.5% and 52% alfalfa hay was added to provide the same levels of TDN. These TDN levels represent calculated TDN values, Morrison (1950). Although the TDN for the medium and low levels approximately 69% and 62% respectively for both corn cobs and alfalfa hay, the crude fiber level differed markedly between the corn cobs and alfalfa hay lots. The fiber content of lot 5 (medium TDN level with corn cobs) was approximately double the basal lot; while lots 6 and 7 (low level of TDN with corn cobs and medium level of TDN with alfalfa hay) were about the same with approximately 3 times the level of fiber of the basal lot. However, the low level of TDN with alfalfa hay (lot 8) possessed almost 4 times the level of fiber of the basal lot.

The number of days on experiment and average daily gains were subjected to the analysis of variance. Differences due to sex were not significant. Therefore the sexes were combined for the subsequent analysis of treatment effects. Table 2 contains the lot means for the feed and growth data.

An increase in the number of days on experiment was observed between treatments. Pigs in the hand-fed lot and lot 8 (62% TDN with alfalfa hay) required a significantly longer ($P<0.05$) feeding period than those fed the basal ration. Increased days on experiment were also observed among the pigs in lots 6 and 7 which received low and medium TDN rations respectively, but the differences were not significant. The pigs in lot 5 (69% TDN with corn cobs) responded similarly to those of the basal lot. Longer feeding periods were reported by Ellis and Zeller (1931–32) and Winters *et al.* (1949) when hogs were limited-fed. Crampton *et al.* (1954) and Bohman *et al.* (1955) obtained similar results by restricting the energy content of the ration with fibrous feeds. No signifi-

TABLE 2. SUMMARY OF FEED AND GROWTH DATA

	Lot 1 Basal 76% TDN	Lot 2 Hand-fed 70% of basal	Lot 3 High protein 76% TDN	Lot 4 Low protein 76% TDN	Lot 5 Corn cobs 69% TDN	Lot 6 Corn cobs 62% TDN	Lot 7 Alf. hay 69% TDN	Lot 8 Alf. hay 62% TDN	LSD[a] .05	LSD[a] .01
No. pigs in lot[b]	7	8	7	8	8	7	8	7		
No. days on experiment, av.	122.28	152.12**	123.00	123.75	121.50	136.86	135.00	191.28**	15.84	21.10
Av. daily gain, lb.	1.42	1.16**	1.44	1.45	1.42	1.28*	1.31	0.88**	0.14	0.18
Av. daily feed consumption, lb.	5.38	4.22	5.40	5.67	6.14	7.15	5.99	6.02		
Av. daily TDN consumption, lb.	4.19	3.29	4.19	4.42	4.30	4.51	4.19	3.79		
Feed per cwt. gain, lb.	384.9	366.7	384.4	400.6	435.2	558.4	463.1	697.8		
TDN per cwt. gain, lb.	299.3	285.6	298.3	312.1	304.9	352.1	324.3	438.8		

[a] Least significant difference.
[b] One pig died in each of lots 1, 3, 6, and 8 during the first 6 weeks of the experiment and they are not included in the experimental results.
* Significant at 5% level.
** Significant at 1% level.

cant differences in number of days on experiment were noted between lots 3 and 4 (high and low protein levels) and the basal lot.

Average daily gains of the pigs were decreased by restricting TDN intake in lots 6, 7 and 8, and feed intake in lot 2. These results concur with observations of Ellis and Zeller (1931–32), Winters *et al.* (1949), Whatley *et al.* (1951), Bohman *et al.* (1953), Crampton *et al.* (1954) and Bohman *et al.* (1955). The average daily gains obtained from the pigs of lots 2 and 8 were significantly less ($P<0.01$) than those of the basal lot. A less pronounced decrease was found among the pigs in lots 6 and 7. However, the daily gains of the pigs in lot 6 were significantly lower ($P<0.05$) than those of the basal lot. No significant differences were observed between lot 5 and the basal lot for the average daily gains of the pigs. Likewise pigs fed the high and low protein rations were not significantly different from those fed the basal ration.

Lots 5 and 7 contained 69% TDN and a similar response to the same TDN level with corn cobs and alfalfa hay was anticipated. However, the performance of the pigs in lot 5 corresponded to the basal lot for number of days on experiment and average daily gains; whereas the pigs in lot 7 exhibited marked differences with the same level of TDN. The pigs of of lot 6 restricted to 62% TDN with corn cobs responded similarly to those in lot 7. As previously discussed, lot 5 contained approximately twice as much fiber as the basal ration, while lots 6 and 7 possessed nearly 3 times as much fiber as the basal ration. Thus, it appeared the effect of crude fiber level was more pronounced than the level of TDN. The response of the pigs in lot 8 which contained 4 times as much fiber as the basal ration was even more marked. Bohman *et al.* (1955) reported the rate of gain was inversely proportional to the crude fiber level in the rations and this appears to be the factor exerting the greatest influence upon the experimental results. Results reported by Axelsson and Eriksson (1953) and Bohman *et al.* (1955) are not in agreement. However, Axelsson and Eriksson (1953) used wheat straw as a source of fiber; whereas the latter fed alfalfa. Bohman *et al.* (1955) postulated that alfalfa may contain a growth inhibiting substance for swine and they attributed these differences to this assumption. Axelsson and Eriksson (1953) stated that for maximum gains the optimum level of crude fiber in the dry matter of the rations averaged 6.57%, but 7.26% fiber was required for the maximum efficiency of metabolizable energy. In studies conducted with oat mill feed for pigs Lathrop and Bohstedt (1938) reported rate of gain and feed consumption were normal up to 7.5% fiber, but beyond this level a marked suppression was noted. Although the fiber content of the ration of lot 5 exceeds this level during the first period, the level approached it in the finishing period. This would tend to indicate that the fiber content of lot 5 did not exceed the tolerance limits for swine, since comparable growth rates were obtained in all self-fed lots containing less than 10% crude fiber. Bohman *et al.* (1955) reported that as the level

of alfalfa in the ration increased, the difference in rate of gain between pigs receiving chlortetracycline and the controls decreased. The addition of Aurofac to the ration in the present study may also partially explain the results observed in lot 5.

Daily feed consumption of the pigs were increased by the medium and low TDN rations, particularly in the lots receiving corn cobs. Probably these pigs consumed more feed in an attempt to compensate for the reduced TDN content. Axelsson and Eriksson (1953) reported an increase in daily feed consumption with increasing fiber levels, indicating that within a certain degree of pigs compensated for the reduced energy content of the rations. This appears evident in the present study, as the daily TDN consumption of the pigs was not markedly different between lots, except lot 2 which was intentionally restricted to approximately 70% of the feed consumption of the basal lot. The average feed consumption of the hand-fed pigs for the entire feeding period was 71.35% of that consumed by the pigs in the basal lot. Daily TDN consumption of the pigs in lot 8 was less than that of the other TDN restricted lots. The incorporation of 52% alfalfa hay into the ration of this lot provided an extremely bulky ration. Consequently it appeared to be physically impossible for the pigs to completely compensate TDN intake by consuming more feed. The pigs in lots 7 and 8 apparently derived some energy from the alfalfa hay, since daily feed consumption was less than the corresponding TDN levels with corn cobs. However, adverse palatability and/or the high fiber content of the "poor quality" alfalfa hay may have reduced daily feed consumption.

Feed efficiency (lb. of feed per lb. gain) decreased at the lower TDN levels and was especially marked among the pigs in lot 8. Some feed wastage occurred in the corn cob and alfalfa hay lots which was calculated as feed consumed. Bohman *et al.* (1955) found that as the level of alfalfa in the ration increased, the amount of feed required per 100 lb. gain increased. The highest feed efficiency was attained by the pigs in the hand-fed lot and the least efficient gains occurred among the pigs of lot 8. Since the digestible portion of the rations varied considerably between treatments in this study, the amount of TDN required per 100 lb. of gain was calculated. With the exception of the pigs in lot 8, essentially little or no difference in TDN per 100 lb. gain was found between lots. Winters *et al.* (1949) observed little difference in the TDN consumed per pound of gain with hogs restricted in feed intake. Although daily TDN consumption of the pigs in lot 8 was less than the other lots, except for lot 2, the TDN required per 100 lb. gain was highest in this lot. This was reflected in the low daily gain of these pigs, which resulted in the greatest number of days on experiment. The feed efficiency of the pigs in lot 3 (high protein) and the basal lot was identical, but the pigs receiving the low protein ration required slightly more TDN per 100 lb. gain, probably because the protein level was marginal.

The total cost per 100 lb. of gain was highest among the pigs in the low (62%) TDN lots and the hand-fed lot. Feed costs probably reflected the increased number of days on experiment with the low TDN rations; whereas the hand-fed lot also included additional labor costs. Whatley *et al.* (1951) reported increased cost of gains with pigs restricted in energy intake. Feed costs of the other lots were approximately the same.

It has been suggested in the discussion thus far that the level of crude fiber exhibited a more pronounced influence upon the number of days

TABLE 3. CORRELATION COEFFICIENTS OF TDN, FIBER AND PROTEIN LEVELS WITH GROWTH DATA AND CARCASS CHARACTERISTICS

	TDN	% Fiber	% Protein
	r	r	r
No. days on experiment	—.709*	.872**	—.272
Av. daily gain	.764*	—.908**	.291
Dressing %	.819*	—.960**	.314
Av. fatback thickness	.741*	—.904**	.224
Grade[a]	.691	—.873**	.205
% Lean cuts[b]	—.749*	.907**	—.036
% Ham muscle	—.773*	.859**	—.024
Area of longissimus dorsi muscle	—.348	.457	.417
Area of ham butt	—.667	.683	.114
Length snout to hind foot	—.748*	.857**	—.576
Length 1st rib to aitch bone	—.711*	.855**	—.438
Length of hind leg	—.682	.781*	—.366
Length of ham	—.855**	.888**	—.529
% Viscera[c]	—.838**	.963**	—.328
Leaf fat weight	.616	—.678	.257
% Liver	—.080	—.088	.404
Iodine number	—.512	.635	.238

TDN=Total digestible nutrients; r=Correlation coefficient.
* Significant at 5% level.
** Significant at 1% level.
[a] Grades were coded as follows: Cull=1, Medium=2, Ch. I=3, and Ch. II=4.
[b] Includes ham, loin, picnic shoulder and Boston butt.
[c] Includes contents.

on experiment and average daily gain than either TDN level or protein content. The results obtained with controlled high and low protein rations would tend to substantiate these effects for level of protein. However, simple correlation coefficients were determined between the lot averages for TDN, crude fiber and protein level, and lot means of the growth data and carcass characteristics. The results of the hand-fed lot were excluded from the calculation of the correlation coefficients. The correlation coefficients appear in table 3.

The correlation coefficients between TDN level and the number of days on experiment, and average daily gain (—.709 and .764 respectively) were significant. However, highly significant correlation coefficients (.872 and

—.908 respectively) were obtained between percent crude fiber and the number of days on experiment and average daily gain. The number of days on experiment and average daily gains were not significantly correlated with the level of protein in the ration.

Dressing percentages and average fatback thichnesses were significantly correlated (.819 and .741 respectively) with TDN level, but the percent crude fiber was highly significantly correlated (—.960 and —.904 respectively) with dressing percentages and fatback thicknesses. Carcass grade, although approaching significance at the 5% level, was not significantly correlated with TDN level; whereas a highly significant correlation (—.873) was observed with the crude fiber content of the ration. The areas of the longissimus dorsi muscle and ham butt were not significantly correlated with either TDN or crude fiber level. Likewise, leaf fat weight, percentage liver, and iodine number were not significantly correlated with TDN or crude fiber level. Length of ham and percent viscera were highly significantly correlated with both TDN and fiber level. Length of hind leg was not significantly correlated with TDN level, but a significant correlation coefficient (.781) was found with the level of crude fiber. Since the high fiber lots resulted in a longer feeding period, age may be the influencing factor associated with increased length. The correlation coefficients between the level of TDN and the remaining carcass characteristics were significant; whereas highly significant correlation coefficients were obtained with the crude fiber level and the corresponding carcass characteristics. None of the carcass date examined were significantly correlated with the level of protein in the ration.

Summary

Restricting the TDN level by incorporating fibrous feeds into swine rations significantly decreased average daily gains. Feed consumption per 100 lb. gain was increased by restricting the digestible nutrients of the ration, but TDN consumed per 100 lb. of gain was essentially equal in all lots, except lot 8. Significant differences were observed between the pigs of the basal and hand-fed lot for the number of days on experiment and average daily gain. More efficient gains were also obtained in the hand-fed lot. No significant differences were found between protein levels.

The level of crude fiber in the ration was found to be more highly correlated with the results of growth and carcass data than either TDN or protein level. The differences between treatments were not significant when the crude fiber content of the ration was less than 10%. In lot 8 pig performance was more severely affected by the high level of fiber or low TDN content of the alfalfa hay in the ration than the 70% restriction of the hand-fed lot.

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THE INFLUENCE OF LIMITED FEEDING, USING HIGH FIBER RATIONS, UPON GROWTH AND CARCASS CHARACTERISTICS OF SWINE.[1] II. EFFECTS UPON CARCASS CHARACTERISTICS

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SWINE produce carcasses with less fatback and higher yields of lean cuts when feed consumption is restricted, as reported by Ellis and Zeller (1931–32), Winters *et al.* (1949) and Brugman (1950). Comparable results were observed when the energy content of the ration was restricted with fibrous feeds, Whatley *et al.* (1951), Axelsson and Eriksson (1953), Crampton *et al.* (1954), Coey and Robinson (1954) and Bohman *et al.* (1955). The influence of self-fed, high fiber rations upon carcass characteristics is discussed in this paper.

Experimental

The experimental design and methods used in this study are presented in the preceding paper devoted to discussion of feed and growth data, Merkel *et al.* (1958). In addition to methods already described, the following procedures were employed. Carcass grade was obtained from carcass weight and average fatback thickness, in accordance with U.S.D.A. Pork Carcass Grade Standards, February 1950.

Immediately following the dehairing operation, during slaughter, a muscle sample approximately 1½ inches long, ¾ inch wide and ⅜ inch thick was removed from the left loin (longissimus dorsi), just posterior to the last rib, for subsequent moisture and fat analysis. Moisture was determined by drying in an oven at 95–100° C. at normal atmospheric pressure for 24 hours. Loss in weight was used to calculate percentage moisture. The fat content of the muscle was obtained by extracting the moisture-free sample with diethyl ether for 12 hours. The carcass data were subjected to Snedecor's Analysis of Variance (1950).

Results and Discussion

Lot means and least significant differences appear in table 1. The pigs receiving the basal ration had the highest dressing percentages, while the lowest dressing percentages were obtained from lot 8, followed by lots

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TABLE 1. SUMMARY OF CARCASS DATA

	Lot 1 Basal 76% TDN	Lot 2 Hand-fed 70% of basal	Lot 3 High protein 76% TDN	Lot 4 Low protein 76% TDN	Lot 5 Corn cobs 69% TDN	Lot 6 Corn cobs 62% TDN	Lot 7 Alf. hay 69% TDN	Lot 8 Alf. hay 62% TDN	LSD P .05	LSD P .01
Av. dressing %	73.11	72.85	72.74	73.02	72.11	69.96**	68.66**	64.72**	1.69	2.25
% Viscera	13.60	13.55	14.32	14.06	15.06*	16.48**	17.84**	19.77**	1.32	1.76
Av. fatback thickness, in.	1.69	1.64	1.72	1.77	1.76	1.58	1.56	1.17**	0.23	0.30
Grade: Ch. I	6	3	4	4	3	5	6	..		
Ch. II	1	3	2	3	4	..	..	..		
Medium	..	2	1	1	1	1	1	4		
Cull	..	..	..	..	..	..	1	3		
Grade[a]	3.14	3.12	3.14	3.25	3.38	2.83	2.62	1.57**	0.65	0.89
% Lean cuts	45.99	48.62	47.75	45.46	47.24	47.90	49.12*	51.88**	2.90	3.86
% Loin	15.10	15.49	15.10	14.54	15.20	14.51	15.62	16.24	N.S.	N.S.
% Skinned ham	16.07	17.19	16.76	16.14	16.80	17.84**	17.75**	18.86**	1.26	1.68
% Ham muscle	12.28	12.92	12.77	11.62	12.41	13.38	13.85*	13.92**	1.27	1.62
% Ham muscle of regular ham	62.60	64.54	63.54	60.66	63.49	64.39	65.55	63.70	N.S.	N.S.
Area of longissimus dorsi muscle, sq. in.	3.84	4.00	3.82	3.54	3.75	3.78	3.93	3.85	N.S.	N.S.
Area of ham butt, sq. in.	17.73	19.32	19.08	18.02	18.25	19.12	18.63	19.26	N.S.	N.S.
% Belly	15.94	15.05	15.33	16.42	15.41	15.88	15.83	14.43	N.S.	N.S.
Length snout to hind foot, in.	63.22	66.85**	63.77	65.21*	64.71	65.25*	65.73**	67.26**	1.87	2.49
Length 1st rib to aitch bone, in.	28.13	28.86	28.26	28.46	28.52	28.43	28.55	29.10	N.S.	N.S.
Length of hind leg, in.	19.81	21.28**	20.35	20.50	20.14	20.67*	20.81*	21.05**	0.80	1.06
Length of ham, in.	12.29	13.46**	12.56	12.76	12.62	13.12**	12.90*	13.38**	0.59	0.79
Length of neck, in.	6.11	6.20	6.28	6.02	6.24	6.07	6.01	5.78	N.S.	N.S.
Leaf fat weight, lb.	5.08	4.59	4.10	4.09	5.09	3.57*	4.22	3.10**	1.15	1.54
% Liver	1.95	1.69*	1.91	1.59**	1.76	2.00	1.76	1.70*	0.22	0.29
Iodine number	60.27	59.79	62.15	58.82	59.29	61.29	59.74	64.33**	3.04	4.05
Longissimus dorsi muscle:										
% Moisture	73.32	74.75*	73.96	73.68	73.73	75.02*	74.26	75.36**	1.31	1.74
% Fat, FB[b]	4.06	2.47	3.03	4.07	3.61	2.49	2.62	2.71	N.S.	N.S.
% Fat, MFB[c]	15.10	9.55	11.35	15.38	13.40	9.89	9.94	10.93	N.S.	N.S.

LSD=Least significant difference; N.S.=Not significant.
* Significant at 5% level.
** Significant at 1% level.

[a] Code values: Cull=1, Medium=2, Ch. I=3, and Ch. II=4.
[b] FB=Fresh basis.
[c] MFB=Moisture free basis.

6 and 7. Dressing percentages of the pigs were significantly less ($P<0.01$) than the basal lot in lots 6, 7 and 8. The dressing percentages of the pigs from the hand-fed lot, the high and low protein lots and lot 5 (69% TDN with corn cobs) were not significantly different from those of the basal lot. These data would tend to indicate that the reduction in dressing percentage was apparently associated with the crude fiber content of the ration. This appears evident, since the pigs in the hand-fed lot, which was limited to 70% of the feed intake of pigs receiving the basal ration, produced dressing percentages comparable to those of the control pigs. These results concur with the observations of Whatley *et al.* (1951), Axelsson and Eriksson (1953), Coey and Robinson (1954) and Bohman *et al.* (1955).

The percentage viscera (plus contents) was significantly higher ($P<0.05$ in lot 5 and $P<0.01$ in lots 6, 7 and 8) than from the pigs of the basal lot. In contrast to most carcass characteristics, the pigs from the hand-fed lot did not differ significantly from those of the basal lot for percentage viscera. In addition, no significant differences were observed between the pigs from the high and low protein lots and the basal lot. Bohman *et al.* (1953) observed a marked enlargement of the stomach and intestines of pigs having received 30% and 50% alfalfa. Coey and Robinson (1954) found increased offal weights as the fiber content of pig rations increased from 3.5% to 11.0%. They indicated the increases were probably due to heavier large intestines. In a second study, Bohman *et al.* (1955) reported the stomach and large intestine of hogs fed 50% alfalfa were 22% and 32% heavier than controls (no alfalfa). They observed no change in size or weight of the small intestine. An inverse relationship was evident between percentage viscera and dressing percentage in the present study. No separation of the different anatomical components of the viscera was made. The percentage viscera increased as the crude fiber content of the ration increased; however, no data were collected for the empty viscera, consequently the percentages reported for viscera in this study cannot be designated as being due to either visceral contents or viscera weight per se.

Average fatback thicknesses of the carcasses in lot 8 were significantly less ($P<0.01$) than those of the basal lot. No significant differences were found between the carcasses of the other lots, but a slight decrease in fatback thicknesses was observed in lots 6 and 7. The fatback thickness of each carcass is reflected in carcass grade. The number of carcasses in each grade is indicated for all lots in table 1.

The largest number of choice No. 1 carcasses was found in the basal lot, and lots 6 and 7. Even though grades and fatback thicknesses were quite comparable in these three lots, the carcasses obtained from lots 6 and 7 were decidedly leaner than those from the basal lot as evidenced by the percentage lean cuts. Mansfield and Trehane (1935) and Crampton *et al.* (1954) reported leaner and higher grading carcasses from

[c] MFB = Moisture free basis.

** Significant at 1% level.

moderately restricted-fed hogs. The carcasses from lot 8 graded the lowest. It should be emphasized that the carcasses were graded solely on carcass weight and average fatback thickness, and only in lot 8 were the medium and cull carcasses definitely inferior in quality. Factors which probably contributed to the low quality carcasses observed in lot 8 are discussed later. Lots 2, 4 and 5 produced somewhat fatter carcasses and a larger number of choice No. 2 carcasses were obtained.

Results obtained by coding carcass grades (see table 1) paralleled the average fatback thicknesses. The coded grades of the carcasses from lot 8 were significantly lower ($P<0.01$) than those of the basal lot. Similarly actual carcass grade and fatback thicknesses of lot 8 were markedly less than carcasses from other lots. No significant differences for coded grades were observed between the remaining lots. Thus, the digestable nutrient content of the rations in lots 6 and 7 was consistent with production of high grading carcasses, but apparently the level of restriction in lot 8 was too severe as evidenced by inferior quality, low grading carcasses.

Gilts were significantly leaner than barrow carcasses. The individual and combined lean cuts as well as the area of the longissimus dorsi muscle were significantly higher for gilts. Similar results were obtained by Whatley *et al.* (1951) from carcasses of pigs fed low energy rations.

The percentages of lean cuts from the carcasses of the restricted-fed lots exceeded those of the basal lot, but the increases in carcass leanness were only significant in lots 7 and 8 ($P<0.05$ and $P<0.01$ respectively). The lowest yield of lean cuts was obtained from the carcasses of the basal and low protein lots. Ashton *et al.* (1955) reported increased percentages of lean cuts as the level of protein in the ration increased. The high protein lot in this study produced carcasses with higher percentages of lean cuts than the low protein lot, but the increases were not significant. Higher percentages of lean cuts were obtained from the carcasses of the hand-fed and corn cob lots than from the basal lot; however, the differences were not significant. Carcass quality in these two lots was decidedly superior to lot 8. The leanest carcasses were produced in lot 8, but carcass quality and grade, as already discussed, were inferior to the other lots.

The percentage loin and area of the longissimus dorsi muscle were not significantly different between treatments. On the other hand, the percentages of skinned ham were significantly higher ($P<0.01$) in lots 6, 7 and 8 than from the carcasses of the basal lot. Likewise, ham muscle expressed as percentages of the total carcass weight, was increased in these lots, and the increases were significantly higher ($P<0.05$ and $P<0.01$) in lots 7 and 8 respectively. The lowest percentages of skinned ham and ham muscle were produced by the carcasses from the basal and low protein lots. The percentages skinned ham and ham muscle paralleled the results observed between treatments for the combined lean cuts. Even though the percentages of skinned ham and ham muscle were significantly influenced by treatment, no significant differences in muscle area of the

ham butt or percentage separable lean of the regular ham were obtained between lots. Percentage belly was not significant between treatments.

Length of the carcasses from snout to hind foot in all lots exceeded those of the basal lot. The differences in length of the carcasses were significant at the $P<0.05$ level in lots 4 and 6, and $P<0.01$ level in lots 2, 7 and 8. The longest carcasses were observed in lot 8. Length from first rib to the aitch bone was slightly greater in the carcasses of restricted-fed lots, but the increases were not significant. Burroughs and Carroll (1939) and Brugman (1950) reported slightly longer carcasses from restricted-fed pigs. The restricted-fed pigs required a longer feeding period to reach slaughter weight; thus, age may be a factor contributing to increased length.

The hind leg of the carcasses was significantly longer ($P<0.05$ in lots 6 and 7, and $P<0.01$ in lots 2 and 8 respectively) than those of the basal lot. Burroughs and Carroll (1939) noted a slight increase in length from the aitch bone to the rear toe in pigs restricted to 75% of full feed. Thus, it would appear from these data that length of hind leg accounted for the increased length from snout to hind foot. However, further confinement of this measurement to the length of ham produced results comparable to the intact hind leg. The increases in length of ham of the carcasses was significant at $P<0.05$ level in lot 7 and $P<0.01$ in lots 2, 6 and 8. Apparently then, the increased length from snout to hind foot was intensified in the length of ham. No significant differences in length of neck were obtained between lots.

In the above discussion of lean cuts it was observed that the percentages of skinned ham and ham muscle were significantly increased; whereas percentage loin was not significantly different between treatments. Likewise, length of ham was significantly increased, but no significant differences were found between lots for the length from the first rib to the aitch bone (essentially the length of the loin), or the muscle areas of the ham butt and longissimus dorsi muscles. These data would tend to indicate that length of ham was more highly associated with percentage skinned ham than area of muscles in the ham butt. Neither longissimus dorsi muscle area nor length from the first rib to the aitch bone were increased sufficiently by treatment to significantly influence the percentage loin.

The weight of leaf fat from the pigs of lots 6 and 8 was significantly less ($P<0.05$ and $P<0.01$ respectively) than those of the basal lot. Essentially no differences in weight of leaf fat were observed between the pigs of lot 5 and the basal lot. Pigs fed the high and low protein levels yielded similar weights of leaf fat, but somewhat less than those fed the basal ration; however, differences were not significant. Likewise no significant differences were found between carcasses from the hand-fed and basal lots. These data indicate that leaf fat was not entirely concurrent with carcass fatback deposition.

Although percentage liver was significantly less ($P<0.05$ in lots 2 and 8, and $P<0.01$ in lot 4) than from pigs fed the basal ration, the decrease was not proportional to the TDN or fiber level, but was more closely associated with the protein content of the rations. Carroll *et al.* (1935–36) observed a direct association between liver weight of hogs and the level of protein in the ration.

Fatback from the carcasses of lot 8 possessed a significantly higher ($P<0.01$) iodine number than those of the basal lot. No significant differences were observed between the remaining lots. These data further substantiate the inferior quality of the carcasses from lot 8. Higher iodine numbers were reported by Ellis and Zeller (1931–32), St. Pierre *et al.* (1934) and Coey and Robinson (1954) for the fat of restricted-fed pigs. Even though the iodine number of the fatback from the pigs in lot 8 was highest, the fat was markedly softer and much more oily than the other lots.

The moisture content of the longissimus dorsi muscles was significantly higher ($P<0.05$ in lots 2 and 6, and $P<0.01$ in lot 8) than the basal lot. The high moisture content of the muscle tissue from the carcasses of lot 8 probably contributed to the lack of quality observed in this lot. The effect of treatment upon the fat content of the longissimus dorsi muscle, expressed either on a fresh or moisture-free basis, was not significant. However, the highest and lowest percentages of intra-muscular fat were obtained in the basal and restricted-fed lots respectively.

Summary

The incorporation of the fibrous feeds, corn cobs or alfalfa hay, into swine rations significantly decreased dressing percentages, carcass fatback thicknesses and leaf fat weights. The percentages of skinned ham, ham muscle and lean cuts were increased in the carcasses of restricted-fed pigs. The highest grading carcasses were obtained from pigs fed the basal ration and those fed 62% and 69% TDN with corn cobs and alfalfa hay respectively. However, the pigs from the latter two lots were leaner and yielded more desirable carcasses than those fed the basal ration. Gilts produced significantly leaner carcasses than barrows. No significant differences were observed between treatments for areas of the longissimus dorsi muscle, ham butt or the percentage belly.

Length from snout to hind foot, length of hind leg and length of ham were significantly longer in the carcasses from restricted-fed pigs. No significant differences for length of carcass were observed between treatments. The percentages of viscera from pigs fed the lower TDN levels were significantly higher than those from the basal lot. The moisture content of the longissimus dorsi muscle and the iodine number of the fatback from carcasses of pigs fed 62% TDN with alfalfa hay exceeded all other lots and they were significantly higher than pigs fed the basal

ration. Intra-muscular fat was not significantly affected by treatment. The leanest carcasses were produced from pigs fed 62% TDN with alfalfa hay; but carcass quality and grade were decidedly inferior to other lots.

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SOME SIMPLE CUT INDICES FOR PREDICTING CARCASS TRAITS OF SWINE. I. CUT-OUT AND LOIN LEAN AREA [1]

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A NUMBER of workers have reported that a single wholsale cut may be used as an index to carcass composition (McMeekan, 1941; Hankins and Howe, 1946; Whiteman *et al.*, 1953; Fredeen *et al.*, 1955; Pearson *et al.*, 1956a; Price *et al.*, 1957). Application of the methods has ranged from physical separation to specific gravity determinations. Although these methods are useful research tools, they are not readily adaptable to large scale useage. However, such studies do suggest the possibility of utilizing some simple wholesale cut index as a means of carcass evaluation. Bratzler *et al.* (1947) reported a high relationship between the loin index, which is the percentage of trimmed loin to rough loin, and cut-out values. Although the study has not been reported in detail, results indicated that simple cut indices may be useful in evaluation procedures. Consequently, this study was undertaken to ascertain the usefulness of some simple cut indices in evaluation of pork carcasses.

Relationships were calculated for cut-outs and loin lean area to ascertain the usefulness of each of the simple cut indices in carcass evaluation. The cut indices used were loin index, the trimmed loin fatback ratio, percentage trimmed loin (live and carcass basis), percentage skinned ham (live and carcass basis), percentage New York shoulder (live and carcass basis) and percentage trimmed belly (live and carcass basis).

Materials and Methods

Experimental Animals. The data used in this study were collected from pigs slaughtered during a six year period. Complete records were available on breeding, sex and cut-out. Since some of the pigs came from experiments involving the effects of delayed castration, hormone treatment or limited feeding, which have been described in detail by Soule (1950) and Rust (1953), these treated pigs were placed in a separate group for purposes of statistical analysis. A total of 195 pigs was involved in this study, which included 83 barrows, 62 gilts and 50 treated pigs.

Slaughtering, Cutting and Measuring. Slaughtering, cutting and measuring procedures were essentially the same as those previously reported by Pearson *et al.* (1956a, 1956b). Percentage fat trim was not available for

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hogs slaughtered prior to 1955–56, which included all of the treated group.

Statistical Treatment. Correlation coefficients were calculated as outlined by Snedecor (1946). Because of possible differences due to sex and/or treatment, the data were analyzed separately for barrows, gilts and treated pigs. Originally, the three groups were combined to give an overall correlation coefficient, but due to the consistently higher relationships for the treated group, the combined correlation coefficients reported include only barrows and gilts. Although the treated group varied widely in degree of fatness due to effect of age at castration, hormone treatment or restricted feeding, no attempt was made to segregate into sub-classes.

TABLE 1. MEANS AND STANDARD DEVIATIONS BY SEXES AND/OR TREATMENT AND COMBINED

	Means and standard deviations			
	Barrows	Gilts	Treated	Combined[a]
Slaughter wt., lb.	204.8(9.9)	205.9(12.6)	210.9(6.1)	206.7(10.3)
Lean cuts-live, %	36.01(1.73)	36.67(1.80)	37.51(2.51)	36.61(2.06)
Lean cuts-carcass, %	48.08(2.22)	48.89(2.53)	50.89(3.63)	49.05(2.95)
Primal cuts-live, %	47.91(1.88)	48.59(1.86)	49.01(2.26)	48.41(2.02)
Primal cuts-carcass, %	63.97(2.28)	64.84(2.53)	66.54(3.19)	64.90(2.80)
Fat trim, %	26.64(2.78)	26.43(2.57)	.....	26.54(2.67)
Lean area-last rib, sq. in.[b]	4.27(0.54)	4.72(0.59)	4.49(0.71)	4.47(0.63)
Ratio trimmed loin/fatback	1.40(0.26)	1.53(0.29)	1.66(0.72)	1.51(0.44)
Loin index[c]	58.38(4.24)	60.28(4.24)	60.58(6.91)	59.56(5.24)
Trimmed loin-live, %	10.82(0.89)	11.05(0.74)	10.75(0.83)	10.87(0.83)
Trimmed loin-carcass, %	14.44(1.12)	14.75(1.02)	14.56(1.19)	14.57(1.11)
Skinned ham-live, %	12.64(0.75)	13.00(0.86)	13.92(0.94)	13.09(0.98)
Skinned ham-carcass, %	16.87(1.04)	17.36(1.14)	18.88(1.29)	17.54(1.40)
N.Y. shoulder-live, %	12.56(0.68)	12.62(0.69)	12.84(1.00)	12.65(0.79)
N.Y. shoulder-carcass, %	16.77(0.85)	16.84(0.90)	17.43(1.50)	16.97(1.10)
Trimmed belly-live, %	11.93(0.79)	11.91(0.82)	11.50(0.73)	11.81(0.81)
Trimmed belly-carcass, %	15.91(0.99)	15.89(1.12)	15.59(0.89)	15.82(1.02)

[a] Combined sexes includes all pigs.
[b] Lean area includes both *Longissimus dorsi* and *Multifidis dorsi* muscles.
[c] Loin index $= \frac{\text{trimmed loin}}{\text{rough loin}} \times 100$.

Results and Discussion

Means and standard deviations for all measurements are given in table 1. The correlation coefficients are given for each group separately in table 2, while table 3 gives the combined relationships.

A comparison of the correlation coefficients by groups reveals that the treated group consistently had higher relationships between cut-out and single cut indices than either barrows or gilts. One notable exception was the lower association for the treated group between percent belly-live basis and primal cuts-live basis (table 2). Otherwise, the treated group always had an equal or higher relationship for all traits studied than the other two

groups. Examination of the standard deviations by groups (table 1) shows a greater variability for the treated animals and may explain the higher correlation coefficients. This would indicate the need for considering the more normal population of barrows and gilts in evaluation procedures.

TABLE 2. CORRELATION COEFFICIENTS FOR VARIOUS SIMPLE CUT RELATIONSHIPS WITH CARCASS CUT-OUTS AND LEAN AREA OF THE LOIN

Trait	Loin index			Trimmed loin/fatback ratio		
	Barrows[a]	Gilts[b]	Treated[c]	Barrows[a]	Gilts[b]	Treated[c]
Number of carcasses	83	62	50	83	62	50
Lean cuts-live	.80	.63	.81	.74	.60	.77
Lean cuts-carcass	.84	.75	.93	.78	.70	.87
Primal cuts-live	.68	.60	.69	.64	.61	.64
Primal cuts-carcass	.77	.77	.87	.72	.73	.82
Fat trim	—.78	—.80	...	—.77	—.73	...
Lean area-last rib	.57	.48	.64	.57	.44	.61
	Percent trimmed loin-live			Percent trimmed loin-carcass		
Lean cuts-live	.78	.72	.88	.74	.63	.85
Lean cuts-carcass	.66	.70	.80	.74	.74	.88
Primal cuts-live	.70	.74	.84	.62	.65	.76
Primal cuts-carcass	.58	.69	.73	.64	.76	.82
Fat trim	—.49	—.64	...	—.58	—.65	...
Lean area-last rib	.66	.58	.72	.61	.54	.65
	Percent skimmed ham-live			Percent skinned ham-carcass		
Lean cuts-live	.72	.79	.90	.59	.73	.89
Lean cuts-carcass	.73	.70	.79	.74	.77	.91
Primal cuts-live	.64	.64	.90	.47	.58	.83
Primal cuts-carcass	.67	.53	.78	.69	.63	.90
Fat trim	—.67	—.58	...	—.70	—.61	...
Lean area-last rib	.20	.45	.75	.09	.43	.71
	Percent N.Y. shoulder-live			Percent N.Y. shoulder-carcass		
Lean cuts-live	.73	.84	.90	.64	.78	.81
Lean cuts-carcass	.62	.72	.91	.72	.81	.92
Primal cuts-live	.67	.74	.83	.52	.67	.71
Primal cuts-carcass	.55	.59	.87	.64	.72	.89
Fat trim	—.48	—.60	...	—.57	—.68	...
Lean area-last rib	.33	.31	.69	.22	.29	.60
	Percent belly-live			Percent belly-carcass		
Lean cuts-live	—.06	—.16	—.46	—.16	—.19	—.54
Lean cuts-carcass	—.22	—.18	—.60	—.19	—.09	—.55
Primal cuts-live	.38	.29	—.18	.25	.24	—.30
Primal cuts-carcass	.20	.26	—.41	.26	.36	—.33
Fat trim	.17	.07	...	.10	.02	...
Lean area-last rib	.15	.10	—.42	.06	.10	—.52

[a] Barrows require a "r" value of .217 for significance at 5% level and .283 at 1% level.
[b] Gilts require a "r" value of .250 for significance at 5% level and .325 at 1% level.
[c] Treated group requires "r" value of .273 for significance at 5% level and .354 at 1% level.

Consequently, the correlation coefficients in table 3 include only combined values for barrows and gilts, thereby permitting a comparison of the various single cuts using a more representative market swine population.

A comparison of the various indices reveals that the loin index is generally superior to any other measure studied for predicting cut-outs and loin lean area. This is particularly noticeable when a comparison of all correlation coefficients is made for each of the indices. Although this was true for an overall comparison, it was not always true for any single measure of cut-out. For example, the "r" value for loin index and lean

TABLE 3. SUMMARY OF COMBINED CORRELATION COEFFICIENTS [a] BETWEEN VARIOUS SIMPLE CUT RELATIONSHIPS WITH CARCASS CUT-OUTS AND LOIN LEAN AREA [b]

Relationship	Lean cuts live	Lean cuts carcass	Primal cuts live	Primal cuts carcass	Fat trim	Loin lean area
Loin index	.75	.81	.65	.77	—.78	.54
Trimmed loin/fatback ratio	.67	.74	.62	.73	—.75	.51
Loin-live basis, %	.75	.67	.71	.62	—.55	.62
Loin-carcass basis, %	.69	.74	.63	.69	—.61	.58
Ham-live basis, %	.75	.72	.64	.61	—.62	.30
Ham-carcass basis, %	.64	.76	.51	.67	—.65	.23
N.Y. shoulder-live basis, %	.77	.66	.69	.56	—.53	.32
N.Y. shoulder-carcass basis, %	.70	.76	.58	.67	—.62	.25
Belly-live basis, %	—.10	—.20	.34	.22	.12	.13
Belly-carcass basis, %	—.18	—.15	.25	.30	.06	.08

[a] Combined correlation coefficients are based on the barrow and gilt data.
[b] A "r" value of .159 is required for significance at 5% level and .208 at 1% level.

cuts-live basis was +.75, while the New York shoulder-live basis and lean cuts on the live basis was +.77. However, the association between loin index and lean cuts-carcass basis was +.81 compared to +.66 for the corresponding relationship with New York shoulder-live basis (table 3). Thus, it can be concluded that the higher association for percentage New York shoulder-live basis and lean cuts-live basis is due to the part to whole relationship. Close examination of the data (table 3) reveals that the percentage of single cuts and cut-outs are always more closely associated when placed on the same basis, i.e. skinned ham-live basis is more closely associated with lean cuts on the live basis than on the carcass basis, while for skinned ham-carcass basis, the reverse is true.

The trimmed loin-fatback ratio and percentage skinned ham on both the live and carcass basis appeared to be about equally useful in predicting all cut-outs and loin lean area. Although skinned ham and New York shoulder (both bases) reflected cut-out about as accurately as the loin indices, they were distinctly inferior as indicators of loin lean area. Thus, it can be concluded that the trimmed loin-fatback ratio and percentage trimmed loin (both bases) are better measures of carcass muscling than

the skinned ham (both bases) or New York shoulder (both bases), and thus appear to more accurately reflect carcass desirability.

In general all relationships of percentage belly on either the live or carcass basis were quite low. This would indicate that the belly is a rather poor measure of leanness or fatness. Percent belly on both the live and carcass basis was negatively correlated with lean cuts regardless of the basis of expression. Thus, the conclusions of the North Central Livestock Marketing Research Committee (1952) that the belly is negatively correlated with lean cuts is verified. On the other hand, the relationships between percentage belly (both bases) and primal cuts (both bases) are all positive for the combined group (table 3). However, examination of the data in table 2 for the carcasses by groups reveals variability in consistency and direction of relationships. Thus, it can be stated that the belly, being a fat cut, is being selected against if selections are made on the basis of lean cuts. From observations on this and other studies, it is postulated that the use of primal cuts in selection programs may retard progress in development of hogs producing a high proportion of lean meat due to the confounding influence of the belly. On the other hand, the possibility exists that selection for lean cut-outs may result in bellies too thin to satisfy consumer preference. However, assuming that the desired end is production of leaner, meatier pork carcasses and that thin bellies do not become a major problem, it is postulated that total lean cuts may be a sounder approach for selection programs.

Since backfat thickness has been frequently accepted as an excellent measure of carcass cut-out, it was believed advisable to compare backfat with loin index, which was the most accurate simple cut measurement studied, as an index of carcass cut-outs. The associations of backfat thickness to lean cuts-carcass basis for the 195 pigs included in this study were —.51 for the barrows, —.40 for the gilts, —.85 for the treated and —.68 for the over-all combined groups, while corresponding relationships by groups for loin index and lean cuts-carcass basis were +.84, +.75, +.93 and +.84, respectively. Thus, loin index was superior for predicting lean cuts-carcass basis for each group. The correlation coefficients reported in this study for backfat thickness and lean cuts-carcass basis are lower than the —.84 reported by the North Central Livestock Marketing Research Committee (1952) or the —.82 reported by Henning and Evans (1953). On the other hand, relationships of —.72 and —.70 were reported for two groups of pigs by Brown *et al.* (1951); while Whiteman *et al.* (1953) reported —.78 and —.59 for two different groups of pigs, while Pearson *et al.* (1956a) obtained —.49 and Price *et al.* (1957) —.72. The reason for the difference in association between backfat and lean cuts is not clear, but the fact that loin index had a predictive value equal to the highest and superior to the others would indicate loin index to be more reliable than backfat thickness for predicting lean cuts.

Results of this study indicate that various simple cut indices may be

useful tools in carcass evaluation. Over-all results indicate a slight superiority of the loin index with little difference between the trimmed loin-fatback ratio or percentage trimmed loin (either basis). Percentage skinned ham (both bases) and percentage New York shoulder (both bases) were good indicators of cut-out but were poor for predicting muscling. The belly was inferior to all other indices studied.

Summary

Results indicate that simple cut indices involving a minimum number of weights and adapted to large scale useage may be utilized in evaluating swine carcasses. The loin index, which is the percentage of trimmed loin in relation to rough loin, appears to be more promising than the trimmed loin-fatback ratio or the percentage trimmed loin on either the live or carcass basis. A comparison of the loin index and backfat thickness as measures of lean cuts (carcass basis) justifies the conclusion that loin index more accurately reflects percentage lean cuts. Although percentage skinned ham (both live and carcass bases) and percentage New York shoulder (both bases) indicate cut-out nearly as well as the various loin indices, all loin indices were superior as indicators of loin lean area. On the other hand, the percentage trimmed belly was a poor indicator of all cut-outs and loin lean area.

Consideration of the pigs by groups showed the hormone treated-delayed castrated-limited fed group generally had higher correlation coefficients between the cut indexes and all carcass cut-outs and loin lean area than was true for the barrows or gilts. The greater variability of the treated group was apparently responsible for the higher relationships. Data from pigs on treatments which are distinctly different than those commonly used by commercial swine producers should not be used as a basis for measuring the usefulness of various evaluation procedures.

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SOME SIMPLE CUT INDICES FOR PREDICTING CARCASS TRAITS OF SWINE. II. SUPPLEMENTARY MEASURES OF LEANNESS [1]

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THE usefulness of some simple cut indices for predicting carcass cut-outs and loin lean area has been reported previously (Pearson *et al.*, 1958). Since supplementary measures are often used in attempting to ascertain leanness, this study was extended to include the relationships between such measures and the simple cut indices. In addition, the supplementary measures are considered from a standpoint of usefulness in carcass evaluation procedures. The traits studied include carcass length, average backfat thickness, live probe, dressing percentage, specific gravity of the carcass and of a single ham, percent fat trim and the ratio of fat to lean area.

Materials and Methods

A discussion of the animals, slaughtering, cutting and measuring procedures has been reported (Pearson *et al.*, 1956a, 1958). Correlation coefficients were calculated for the various simple cut indices and the supplementary measures of leanness or fatness. In addition, relationships between some of the supplementary carcass traits and carcass cut-outs were determined.

Results and Discussion

Means and standard deviations for supplementary measures of leanness are recorded in table 1, while means and standard deviations for simple cut indices have been previously reported (Pearson *et al.*, 1958). Correlation coefficients for the various indices and the supplementary measures of leanness are given by groups in table 2. Examination of the data by groups (table 2) shows that the treated group generally had a higher relationship between the simple cut indices and the supplementary measures of leanness than was true for barrows or gilts. This is in agreement with results reported previously (Pearson *et al.*, 1958). The higher relationships for the treated group were usually accompanied by higher standard deviations. Since the treated group showed greater variability than

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the normal market swine population, the subsequent discussion is largely concerned with the combined group of barrows and gilts.

Consideration of the various single cut indices (table 3) shows that the loin index and trimmed loin-fatback ratio were more closely associated with the supplementary measures of leanness than the other simple cut indices. However, the loin index and trimmed loin fatback ratio were about equally related to the supplementary measures of leanness, with the major difference being a higher association between loin index and carcass length. A comparison of the relationships of these two indices with backfat thickness, live probe and specific gravity values indicates there is little difference between the two. However, both loin index and trimmed loin fatback ratio are more closely associated with the other measures of leanness (table 3) than percentage loin on either the live or carcass

TABLE 1. MEANS AND STANDARD DEVIATIONS BY SEXES AND/OR TREATMENT AND COMBINED GROUPS

	Means and standard deviations			
	Barrows	Gilts	Treated	Combined
Carcass length, in.	28.95(1.05)	29.30(0.93)	29.22(0.96)	29.13(1.00)
Av. backfat thickness, in.	1.80(0.19)	1.75(0.19)	1.55(0.25)	1.72(0.23)
Live probe, in.	1.69(0.20)	1.65(0.18)	.....	1.67(0.19)
Dressing percentage	74.86(1.61)	74.95(1.53)	73.74(1.84)	74.60(1.71)
Specific gravity-ham	1.045(0.0079)	1.046(0.0062)	.....	1.046(0.0072)
Specific gravity-carcass	1.030(0.0071)	1.034(0.0055)	.....	1.032(0.0066)
Fat trim, %	26.64(2.78)	26.43(2.57)	.....	26.54(2.67)
Lean area-last rib	4.27(0.54)	4.72(0.59)	4.49(0.71)	4.47(0.63)
Ratio fat/lean area	1.43(0.33)	1.24(0.30)	1.36(0.41)	1.35(0.35)

basis. The major difference appears to be a lower relationship for percentage loin (both bases) with both backfat thickness and live probe than was true for either loin index or trimmed loin-fatback ratio.

Similar to results reported previously (Pearson *et al.*, 1958), percentage ham (both bases) and percentage New York shoulder (both bases) do not have as high a predictive value for the supplementary measures of leanness as do the various loin indices. On the other hand, they reflect most measures of leanness more accurately than percentage of belly (either live or carcass basis).

Consideration of carcass length (table 3) shows that percentage loin (both bases) tends to increase with carcass length. This is of particular interest, since it is a common belief that a longer pig will yield longer, heavier loins and bellies. Although the relationships between carcass length and percentage loin are highly significant, variation in length accounts for only 17 to 18% of the variability in percentage trimmed loin. The relationship between percentage belly (either basis) and carcass length is negligible. Thus, it is apparent that length alone does not increase the percentage of belly. It is postulated that increases in percentage of belly are more likely to be due to an increase in thickness than to length.

TABLE 2. CORRELATION COEFFICIENTS BETWEEN VARIOUS SIMPLE CUT RELATIONSHIPS AND SOME OTHER MEASURES OF CARCASS LEANNESS

Supplementary measures of leanness	Single cut indices														
	Loin index			Trimmed loin/fatback ratio			Percent trimmed loin-live			Percent trimmed loin-carcass			Percent skinned ham-live		
	Barrows[a]	Gilts[b]	Treated[c]	Barrows[a]	Gilts[b]	Treated[c]	Barrows[a]	Gilts[b]	Treated[c]	Barrows[a]	Gilts[b]	Treated[c]	Barrows[a]	Gilts[b]	Treated[c]
Number of carcasses	83	62	50	83	62	50	83	62	50	83	62	50	83	62	50
Carcass length	.45	.34	.64	.36	.27	.58	.56	.16	.47	.55	.22	.53	.16	.14	.43
Backfat thickness	−.60	−.45	−.85	−.61	−.50	−.80	−.29	−.39	−.53	−.37	−.44	−.66	−.33	−.12	−.59
Live probe	−.74	−.64	...	−.73	−.55	...	−.33	−.53	...	−.45	−.54	...	−.61	−.40	...
Dressing percentage	−.05	−.28	−.43	−.01	−.26	−.39	.34	.06	.10	.10	−.24	−.20	−.01	.19	.19
Specific gravity-ham	.68	.67	...	.69	.68	...	.72	.71	...	.74	.68	...	.59	.58	...
Specific gravity-carcass	.75	.71	...	.76	.75	...	.69	.78	...	.73	.76	...	.63	.50	...
Fat trim	−.78	−.80	...	−.77	−.73	...	−.49	−.64	...	−.58	−.65	...	−.67	−.58	...
Lean area-last rib	.57	.48	.64	.57	.44	.61	.66	.58	.72	.61	.54	.65	.20	.45	.75
Ratio fat/lean area	−.68	−.74	−.85	−.67	−.71	−.77	−.61	−.65	−.64	−.59	−.63	−.72	−.12	−.29	−.06
	Percent ham-carcass			Percent N. Y. shoulder-live			Percent N. Y. shoulder-carcass			Percent belly-live			Percent belly-carcass		
Carcass length	.12	.21	.54	.16	.07	.72	.11	.15	.74	−.02	.11	−.37	.06	.19	−.29
Backfat thickness	−.39	−.18	−.76	−.21	−.31	−.77	−.35	−.40	−.84	.26	.23	.53	.20	.15	.42
Live probe	−.69	−.44	...	−.40	−.44	...	−.54	−.50	...	.36	.25	...	.26	.20	...
Dressing percentage	−.34	−.12	−.17	.32	.22	−.14	−.06	−.16	−.42	.35	.06	.45	.05	−.24	.08
Specific gravity-ham	.55	.56	...	.32	.46	...	.33	.47	...	−.08	−.07	...	−.07	−.06	...
Specific gravity-carcass	.61	.50	...	.44	.42	...	.47	.43	...	−.16	−.01	...	−.14	−.00	...
Fat trim	−.70	−.61	...	−.48	−.60	...	−.57	−.68	...	.17	.07	...	.10	.02	...
Lean area-last rib	.09	.43	.71	.33	.31	.69	.22	.29	.60	.15	.10	−.42	.06	.10	−.52
Ratio fat/lean area	−.05	−.30	−.70	.48	.47	.78	.54	.58	.85	−.12	.06	−.63	−.11	.15	−.54

[a] A "r" value of .217 is significant at 5% level or .283 at the 1% level.
[b] A "r" value of .250 is significant at 5% level or .325 at the 1% level.
[c] A "r" value of .273 is significant at 5% level or .354 at the 1% level.

TABLE 3. SUMMARY OF CORRELATION COEFFICIENTS FOR COMBINED SEXES[a] BETWEEN VARIOUS SIMPLE CUT RELATIONSHIPS AND SOME OTHER MEASURES OF LEANNESS[b]

	Supplementary measures of leanness								
Single cut indices	Carcass length	Backfat thickness	Live probe	Dressing percentage	Specific gravity-ham	Specific gravity-carcass	Percent fat trim	Lean area last rib	Ratio fat/lean area
Loin index	.42	—.56	—.70	—.12	.68	.75	—.78	.54	—.70
Trimmed loin/fatback ratio	.32	—.56	—.66	—.12	.68	.76	—.75	.51	—.69
Loin-live basis, %	.42	—.33	—.40	.24	.71	.70	—.55	.62	—.62
Loin-carcass basis, %	.43	—.40	—.48	—.04	.72	.73	—.61	.58	—.61
Ham-live basis, %	.15	—.24	—.53	.05	.59	.60	—.62	.30	—.18
Ham-carcass basis, %	.15	—.31	—.60	—.25	.56	.59	—.65	.23	—.14
N.Y. shoulder-live basis, %	.13	—.24	—.40	.29	.36	.39	—.53	.32	—.51
N.Y. shoulder-carcass basis, %	.12	—.36	—.52	—.10	.37	.43	—.62	.25	—.50
Belly-live basis, %	.03	.25	.30	.25	—.06	—.08	.12	.13	—.01
Belly-carcass basis, %	.03	.18	.22	—.06	—.05	—.05	.06	.08	.03

[a] Combined sexes includes barrows and gilts.
[b] A "r" value above .159 is significant at 5% level and above .208 is significant at the 1% level.

It is evident that (table 3) carcass length is without significant influence on percentage trimmed ham or percentage New York shoulder. Therefore, the low relationships between carcass length and cut-outs (table 4) would be expected.

The loin index and the trimmed loin-fatback ratio are more closely related to backfat thickness and live probe than to any of the other simple cut indices (table 3). However, this may be due to the part to whole relationship, since the association is lower for percentage trimmed loin, in which backfat is not involved directly in the computation. The correlation coefficients in Table 4 show live probe to be more closely related to carcass cut-outs than backfat thickness, thus verifying the results of Hazel and Kline (1952). Even though live probe and backfat are seemingly measures of the same trait, it is apparent that live probe more accurately reflects leanness or fatness (tables 3 and 4) then does backfat thickness.

TABLE 4. THE RELATIONSHIPS OF CARCASS LENGTH, BACKFAT THICKNESS AND LIVE PROBE TO CARCASS CUT-OUTS [a]

Trait [b]	Carcass length	Backfat thickness	Live probe
Lean cuts-live	.42	—.38	—.55
Lean cuts-carcass	.33	—.47	—.61
Primal cuts-live	.33	—.25	—.38
Primal cuts-carcass	.36	—.39	—.38
Fat trim	—.32	.52	.64

[a] Combined barrows and gilts.
[b] A "r" value for significance is .159 at 5% level and .208 at 1% level.

Since specific gravity and chemical analysis have been shown to be closely related (Brown *et al.*, 1951; Whiteman *et al.*, 1953; Price *et al.*, 1957; Liuzzo *et al.*, 1957), the relationship of the various indices and specific gravity is of particular interest. Thus, it is significant that all loin indices are about equally related to specific gravity values and are definitely superior to the other indices investigated (table 3). Higher relationships between loin lean area and the loin indices tends to show that the various loin indices are better measures of muscling. However, the possibility exists that the higher relationship of the loin lean area and the loin indices are simply an expression of the part to whole relationship.

The ratio of fat to lean area (Pearson *et al.*, 1956b) is more closely correlated with the loin indices than with any of the other measures. The loin index and the trimmed loin-fatback ratio appear to be slightly superior to the other two loin indices. Interestingly, the New York shoulder (both bases) is much more closely associated with the ratio of fat to lean area than is percentage ham or belly, but the reason is not readily apparent.

Percentage belly-live basis was positively correlated with dressing per-

centage, backfat thickness, live probe and fat trim (table 3). This would indicate that dressing percentage tends to go up as percentage of belly-live basis increases. The positive relationship of percentage belly with dressing percentage as well as with the usual measures of fatness, such as backfat thickness, live probe and fat trim, indicates that some basis exists for the theory that dressing percentage increases with fatness. As further verification, correlation coefficients were calculated for dressing percentage and each of the fat measures (table 5) with relationships for the combined group (barrows and gilts) of +.20 for backfat thickness, +.28 for live probe and +.19 for fat trim. Although the degree of association of dressing percentage with these measures of fatness is not high, the consistent positive relationships verify that dressing percentage increases with fatness, which is in agreement with the results of Cummings and Winters (1951). This would indicate that yield or dressing percentage, if disregarded entirely, would slowly decrease as leanness increases.

TABLE 5. CORRELATION COEFFICIENTS BY GROUPS FOR DRESSING PERCENTAGE WITH MEASURES OF FATNESS

	Barrows	Gilts	Treated	Combined[a]
Backfat thickness	.22*	.19*	.47**	.20*
Live probe	.40**	.09	...	.28**
Percentage fat trim	.27*	.07	...	.19*

[a] Combined group includes only barrows and gilts.
* Significant at 5% level.
** Significant at 1% level.

Summary

Results of this study indicate that the various loin indices, especially the loin index and the trimmed loin-fatback ratio, reflect the various measures of leanness or fatness more accurately than percentage ham, percentage New York shoulder or percentage belly. Some evidence is presented which suggests the percentage of trimmed loin may reflect true leanness somewhat more accurately than either of the other loin indices. Percentage skinned ham and percentage New York shoulder were more accurate indicators of the supplementary measures of leanness than percentage belly, which was of little value.

Data are presented showing that carcass length and percentage loin are positively correlated, yet variation in length accounted for only 17 to 18 of the variability in percentage loin. Carcass length did not appear to influence percentage belly, percentage ham or percentage New York shoulder, which explains the low relationship between carcass length and cut-outs. Evidence is presented showing some basis for the assumption that dressing percentage increases with fatness. However, the association between various measures of fatness and dressing percentage is low.

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EFFECT OF CHLORTETRACYCLINE ON INCIDENCE OF CERVICAL ABSCESSES AND WEIGHT GAINS OF SWINE

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IN 1954, cervical abscesses ranked third as a cause of condemnation of pork and accounted for 2.6% of all swine condemned upon federal inspection at slaughter (Meat Inspection Branch, ARS, 1954). Of the approximately 50,000,000 swine that were federally inspected in that year, about 1,300,000 heads were condemned due to cervical abscesses and this resulted in a loss of nearly 1 million dollars to the meat packers. Since that time, an increase in the number of cervical abscesses has been reported.

The incidence of cervical abscesses (also known as jowl abscesses, feeder boils, or strangles) may vary on a herd basis from less than 20% to more than 90% (Merck Vet. Manual, 1955). Beta hemolytic streptococci are considered the principal etiologic agent although *Pasteurella multocida, E. coli,* and *Corynebacterium pyogenes* have also been isolated (Collier, 1951; Merck Vet. Manual, 1955: Newsom, 1937; Smith, 1956; Snoeyenbos *et al.,* 1952; Stafseth and Clinton, 1941). It is believed that the causative organisms gain entrance to the body through the mouth. The condition has been reproduced by adding organisms to the feed and to the drinking water (Gouge *et al.,* 1957; Smith, 1956). The abscesses that are produced can be either single or multiple, and may range from 1 cm. to more than 10 cm. in diameter. The abscesses may be near the surface in the jowl area and easily visible or deep seated where they remain undetected until slaughter.

The first indication that cervical abscesses could be controlled by the continuous feeding of chlortetracycline was made by Eggert *et al.* (1955) in an experiment conducted in Iowa. Twenty-five gm. or more of this antibiotic per ton of feed, used from 25 lb. of body weight to market, prevented the occurrence of visible abscesses. Lesser amounts of chlortetracycline were not effective in controlling the infection since 80% of the pigs in a group that received 12.5 gm. per ton of this antibiotic were

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affected. To further investigate these observations, studies were undertaken on a farm in Illinois where visible cervical abscesses were observed in approximately 200 pigs of a herd of 300 in the spring of 1955.

Materials and Methods

A series of four experiments were conducted. The weanling pigs used in the fall-1955 experiments and spring-1956 experiments were from the same group of sows. The pigs of a litter that were used in the various

TABLE 1. COMPOSITION OF BASAL RATION

Experiments I and II Experiments III and IV	Weaning to 50 lb. to 75 lb.	50 to 100 lb. 75 to 125 lb.	100 to 150 lb. 125 lb. to market	150 to 200 lb.
	lb.	lb.	lb.	lb.
Ingredients				
Gr. yellow corn	1462	1595	1694	1836
Meat and bone scraps (50%)	50	50	50	50
Dried whey	50	...	...	...
Soybean oil meal (44%)	400	265	165	30
Dehydrated alfalfa meal (17%)	...	50	50	50
Ground limestone	12	10	6	10
Steamed bone meal	10	15	20	10
Iodized salt	10	10	10	10
Trace mineral premix	3	2	2	2
Vitamin supplementation (per ton)				
Vitamin A, I.U.	4 million	.....	.....	.....
Vitamin D_3, I.U.	800,000	600,000	600,000	480,000
Riboflavin, gm.	4.0	1.5	1.5	1.2
Calcium pantothenate, gm.	6.0	5.0	5.0	4.0
Niacin, gm.	20.0	12.0	12.0	9.6
Choline chloride, gm.	50.0	100.0	100.0	80.0
Vitamin B_{12}, mg.	20.0	10.0	10.0	8.0

experiments were distributed equally according to size to three experimental groups. Individual pig weights were taken at the start of each experiment and on the day before slaughter. Interim weights were taken also at the time that the rations were changed when the pigs weighed approximately 50, 100 and 150 lb. during experiments I and II, and at 75 and 125 lb. during experiments III and IV. Complete ration, as shown in table 1, was fed in all experiments and the total feed consumed by each group was recorded. Clinical observations of all pigs for the presence of abscesses were made at each weighing. The head and jowls of each pig were examined at slaughter for the presence of cervical abscesses. Bacteriologic examination was made of the contents of 3 abscesses during experiments I and II, and of all abscesses found at the termination of experiments III and IV.

In experiment I, 106 fall-farrowed pigs, approximately 9 weeks of age were divided into 3 groups and self-fed either the basal ration or the same ration supplemented with 50 or 100 gm. chlortetracycline per ton for 84 days. Experiment II was a duplicate of experiment I except that it was started 10 days later and with younger pigs because of differences in farrowing dates. Ninety-eight pigs, approximately 7 weeks of age, were divided into 3 groups and self-fed for 109 days the same ration formulations used in experiment I.

In experiment III, 105 spring-farrowed pigs, approximately 8 weeks of age, were divided into 3 groups. One group was self-fed the basal ration while a second group received the basal ration supplemented with 50 gm. chlortetracycline per ton to approximately 75 lb. body weight, then the

TABLE 2. INCIDENCE OF CERVICAL ABSCESSES—EXPERIMENT I

Item	Chlortetracycline (gm./ton feed)			Combined antibiotic groups[a]
	0	50	100	
No. started	35	36	35	71
No. initially infected	8	9	5	14
Head condemnation				
Initially infected	5/8	2/9	2/5	4/14*
Not initially infected	18/27	7/27	7/30	14/57**
Total	23/35	9/36	9/35	18/71**

[a] No significant difference between antibiotic levels—data combined.
* Significantly less than controls $P=0.05$.
** Significantly less than controls $P<0.001$.

antibiotic level was reduced to 20 gm. per ton and fed to the termination of the experiment. The third group of pigs received the basal ration containing 50 gm. chlortetracycline per ton for the duration of the 91-day experiment.

Experiment IV was a duplicate of experiment III with the exception that 114 pigs were allotted to the three experimental groups and continued on experiment for 96 days.

Results and Discussion

Cervical Abscesses. After allotment to the respective treatment groups an examination for initial incidence of cervical abscesses revealed that 21% of the pigs had detectable abscesses. The number of pigs that were infected in the respective groups indicated a uniform initial incidence (table 2) in experiment I. The infection apparently was progressive, or the abscesses were too deep-seated for detection at the start of the experiment since 66% of the heads from the pigs in the control group were condemned at slaughter as compared with an initial incidence of 23%.

No significant difference was found in the incidence of cervical abscesses

at slaughter between the groups of pigs that received the rations supplemented with 50 and 100 gm. chlortetracycline per ton. Therefore, these data were combined for statistical analysis and chi-square test was used. Significantly fewer heads were condemned at slaughter from the two groups of pigs that received the supplemented rations whether the pigs were initially infected ($p=0.05$) or not initially infected ($p<0.001$). This observation would indicate a preventive effect for pigs that were subsequently exposed to the organisms that cause cervical abscesses.

In experiment II (table 3) the distribution of initially infected pigs was not as uniform as that attained in experiment I. The presence of the infection was evident, however, by an over-all incidence in 10% of the pigs. Again the numbers of cervical abscesses in the two groups of pigs

TABLE 3. INCIDENCE OF CERVICAL ABSCESSES—EXPERIMENT II

Item	Chlortetracycline (gm./ton feed)			Combined antibiotic groups[a]
	0	50	100	
No. started	33	33	32[b]	65
No. initially infected	7	1	2	3*
Head condemnations				
Initially infected	1/7	0/1	0/2	0/3
Not initially infected	11/26	6/32	5/28	11/60*
Total	12/33	6/33	5/30	11/63

[a] No significant difference between antibiotic levels—data combined.
[b] Two pigs died—1 accidental; 1, *P. hemolytica* isolated.
* Significantly less than controls $P=0.05$.

receiving the antibiotic-supplemented rations did not differ significantly so the data were combined and treated statistically. At slaughter, 18% of the pigs in the two antibiotic-supplemented groups that were not initially infected were found to have cervical abscesses that resulted in condemnation of heads. The number of condemnations was significantly fewer ($p=0.05$) than the 42% that had abscesses in the control group.

Since no additional benefit was obtained in experiments I and II from the use of 100 gm. chlortetracycline as compared with the 50 gm. level, this latter level was used continuously in one group while in another group the same level was used to 75 lb. body weight and then reduced to 20 gm. per ton in the two subsequent experiments. In both of these experiments, no cervical abscesses were observed until slaughter. The incidence of cervical abscesses among the two control groups (table 4) was 60 and 61%, and is consistent with the normal incidence occurring on this farm. The continuous feeding of rations containing 50 gm. chlortetracycline per ton prevented the occurrence of cervical abscesses in pigs that were held in pens known to have been occupied previously by infected animals. The 2.9 and 2.7% incidence observed among the pigs receiving the reduced

TABLE 4. INCIDENCE OF CERVICAL ABSCESSES—EXPERIMENTS III AND IV

	Chlortetracycline (gm./ton feed)		
To 75 lb.	0	50	50
75 lb. to market	0	20	50
Experiment III			
No. started[a]	35	35	35
Head condemnations	21	1	0
Experiment IV			
No. started	38	37	39
Head condemnations	23	1	0

[a] One runt pig from each group sacrificed for observation in the latter half of the feeding period; cervical abscesses found in the control pig only.

level of antibiotic was significantly smaller ($p<0.001$) than that observed in the control groups.

The contents of all abscesses found were examined bacteriologically and Beta hemolytic streptococci were isolated from 86%, which is similar to the results obtained in studies by Collier (1956). Failure to recover the organisms at the time of culture from the other abscesses that were examined may have been caused by previous freezing or an overgrowth of *proteus sp.*

Weight Gains and Feed Efficiency. The feeding of rations that were supplemented with 50 and 100 gm. chlortetracycline resulted in consistently heavier pigs when all animals in an experiment were fed for the same number of days and marketed at the same time (table 5). In both experiments I and II, the efficiency of feed conversion was improved by the addition of the antibiotic.

The analysis of co-variance was used to adjust the differences in the

TABLE 5. WEIGHT GAINS AND FEED EFFICIENCY FOR PIGS WITH CERVICAL ABSCESSES

Chlortetracycline (gm. per ton feed)	Experiment I			Experiment II		
	0	50	100	0	50	100
No. started	35	36	35	33	33	32
No. sold	35	36	35	33	33	30[a]
Av. initial weight, lb.	41.9	40.2	36.2	29.9	27.1	27.6
Av. final weight, lb.	177.1	191.6	194.0	201.2	221.1	204.5
Av. daily gains, lb.	1.61	1.82	1.88	1.57	1.78	1.62
Adjusted daily gains, lb.[b]	1.51	1.75*	1.85*	1.60	1.85*	1.69
Av. feed per lb. gain, lb.	3.74	3.54	3.43	3.57	3.55	3.29
Av. days to reach 200 lb.[b]	111	95	91	105	91	99

[a] One death accidental; other *P. hemolytica* isolated.
[b] Based on an adjusted initial weight of 33 lb.
* Significantly greater than controls P=0.05.

starting weight of the pigs to an average initial weight of 33 lb., since the relationship between gains and initial weights was shown to be substantially the same for all treatments. In experiment I, the pigs fed the rations containing 50 gm. chlortetracycline per ton gained 0.24 lb. more per day than the controls, and the 100-gm. group, 0.34 lb. more. The difference of 0.10 lb. gain between the two supplemented groups was not significant therefore the data for the treated groups were combined for statistical analysis. The average daily gains of all treated animals was significantly greater ($p=0.05$) than that observed for the controls. During the interim weight periods, the combined average daily gains of the supplemented groups varied from the control group being 0.39, 0.33 and 0.25 lb. greater from the start of the experiment to 65, 100 and 150 lb. body weight. The weight gains of the abscessed and absecess-free pigs were substantially the same.

In experiment II, there was a significant difference ($p=0.05$) of 0.25 lb. average daily gain between the 50-gm. group and the control group. The difference of 0.09 lb. in average daily gain between the 100-gm. group and the control group was not significant. In this latter group, 30 days before the end of the experiment, reduced feed consumption was observed for 7 days. Temperatures remained normal and bacteriologic examinations failed to reveal the cause.

Increments in weight gains for the interim periods were observed with the exception of the last period from 150 lb. to market weight. The average gains of the two antibiotic-supplemented groups cancelled any difference since the 50-gm. group gained 0.14 lb. more and the 100-gm. group gained 0.13 lb. less than the control group. For the other periods, however, positive differences in average daily gains for the supplemented groups of 0.19, 0.35 and 0.22 lb. were obtained for the time intervals between the start of the experiment and the time required to reach 50, 100 and 150 lb. body weight, respectively.

The pigs in experiment II that were infected with cervical abscesses did not gain as rapidly as abscess-free pigs. Abscess-free pigs in the control group gained 0.23 lb. faster than the abscessed pigs. Antibiotic supplementation apparently influenced the growth of the infected pigs since the abscess-free pigs gained only 0.06 lb. more per day than the abscessed pigs in the supplemented groups. (In this experiment also it was calculated that less time would be needed to reach 200 lb. body weight.)

In both experiment III and IV, an increased average daily gain and an improved feed efficiency was demonstrated by the groups of pigs fed the antibiotic-supplemented rations (table 6). The starting weights were again adjusted to a 33 lb. initial weight by analysis of co-variance. The differences in adjusted average daily gains between the antibiotic-supplemented groups in either of these experiments were not significant.

The 0.19 lb. difference in adjusted daily gain betwen the control group in experiment III and the group that received the 20 gm. level of anti-

biotic approached significance (P=0.06) while the difference of 0.22 lb. for the group that received the higher level of antibiotic was significantly greater (P=0.03). The increased gains of 0.24 and 0.26 lb. observed for the supplemented groups in experiment IV were significantly greater than for the controls having a probability of 0.005 and 0.025, respectively. When the data for both experiments III and IV were combined, the adjusted average daily gains were found to be significantly greater (P=0.001) for each of the supplemented groups as compared with the control groups.

TABLE 6. WEIGHT GAINS AND FEED EFFICIENCY FOR PIGS WITH CERVICAL ABSCESSES

	Chlortetracycline (gm. per ton feed)					
	Experiment III			Experiment IV		
To 75 lb.	0	50	50	0	50	50
75 lb. to market	0	20	50	0	20	50
No. started	35	35	35	38	37	39
No. sold	34[a]	34[a]	34[a]	38	37	39
Av. initial weight, lb.	34.2	38.6	38.3	34.4	35.7	36.5
Av. final weight, lb.	163.9	192.5	194.5	166.8	193.3	194.4
Av. daily gains, lb.	1.43	1.69	1.71	1.39	1.64	1.65
Adjusted daily gains, lb.[b]	1.34	1.53	1.56*	1.35	1.59**	1.61***
Av. feed per lb. gain, lb.	3.46	3.45	3.41	3.61	3.56	3.49
Av. days to reach 200 lb.[b]	125	109	107	124	105	104

[a] One pig from each group removed for observation—cervical abscess observed in controls only.
[b] Based on an adjusted initial weight of 33 lb.
* Significantly greater than controls P=0.03.
** Significantly greater than controls P=0.005.
*** Significantly greater than controls P=0.025.

In both experiment III and IV, differences between the adjusted average daily gain for the antibiotic-supplemented and control groups were observed for each period. The increments were 0.22, 0.24 and 0.03 lb. per day from the start of the experiments to approximate weights of 75 lb., 125 lb. and marketing, respectively. Any differences in average daily gains observed between the two antibiotic groups for each period were small and not significant.

As previously observed in experiment II the presence of cervical abscesses had a depressing effect on weight gains. In considering all abscessed and non-abscessed pigs observed in experiments III and IV, the abscess-free pigs gained 0.10 lb. more per day, a difference which approached significance.

The results of these experiments regarding the weight gains and feed efficiency of swine as affected by antibiotic supplementation are consistent with the published data as reveiwed by Braude *et al.* 1953 and Jukes, 1955.

Summary

A total of 418 pigs from 1955-fall and 1956-spring litters that were farrowed on a farm where cervical abscesses had been a recurring problem were used in 4 experiments. Groups of pigs were self-fed either a basal ration or the basal ration supplemented with 50 or 100 gm. chlortetracycline per ton in the first two experiments. In the subsequent experiments, the groups of pigs were self-fed either the basal ration, or the same ration supplemented with 50 gm. per ton or 50 gm. per ton to 75 lb. body weight followed by 20 gm. per ton to market.

The incidence of cervical abscesses was significantly reduced in pigs fed the antibiotic-supplemented rations when compared with the controls, weight gains were increased, feed efficiency improved, and less time required to reach a weight of 200 lb. No advantage was apparent following incorporation of the higher antibiotic level (100 gm. per ton) as compared with the 50-gm. level in the feed of pigs from weaning to marketing on the control of cervical abscesses, weight gains, or feed efficiency. In all instances, however, continuous use of 50 gm. chlortetracycline per ton of feed produced better results than the lower level in either controlling or preventing cervical abscesses.

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THE INFLUENCE OF CERTAIN ANTIBIOTICS ON THE PALATABILITY OF SWINE RATIONS

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THE nutritional effects of antibiotics in swine rations have been reviewed by Braude *et al.* (1953), Jukes and Williams (1953), Stokstad (1954), Jukes (1955), Cunha (1956), and Maddock and Bracket (1956). The value of certain antibiotics in swine feeds is well established and generally accepted by feed manufacturers and swine producers.

One factor which has not been given much consideration, and which represents an important aspect of the overall problem, is the relationship of antibiotics and ration palatability. Prior to the work reported here, studies by Wallace *et al.* (1956) indicated that feed consumption was reduced when crystalline erythromycin was fed to weanling pigs even at levels as low as 2 mg. per pound of ration. This was an unusual observation, particularly in view of the fact that most studies have shown that antibiotics tend to increase feed consumption.

The objective of this study was to determine to what extent the presence of certain crystalline antibiotics might affect the acceptability of practical rations by growing-fattening swine.

Experimental Procedure

Experiment I. In this experiment 26 pigs of Duroc and Spotted Poland China breeding, averaging initally 128.7 lb., were placed in a partially wooded lot. Yellow ear corn and protein supplement were fed ad libitum. The protein supplement consisted of a mixture of 36 parts tankage, 36 parts soybean oil meal, 25 parts alfalfa leaf meal and 3 parts steamed bone meal. This supplement was offered in four separate feeders, each feeder containing the supplement plus one of four antibiotics added at the rate of 40 gm. per ton of supplement. The four antibiotics involved were chlortetracycline, oxytetracycline, penicillin V, and erythromycin. The consumption of supplement from each feeder was measured, fresh supplement provided, and the feeders were rotated to overcome position preference at the end of each week of the experiment. The duration of the experiment was 6 weeks.

Experiments II and III. The pigs used in Experiment II consisted of 18 purebred Durocs averaging initially 39.8 lb. They had been creep fed a ration containing chlortetracycline. Thus it might be assumed that they had become accustomed to this antibiotic by virtue of having consumed it

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during the nursing period. For this reason a group of pigs were used in Experiment III which had never been fed any antibiotic. The 18 animals used in Experiment III consisted of purebred Durocs, Hampshires, and Spotted Poland Chinas and averaged 26.4 lb. initially. In other respects the two experiments were conducted in a similar manner. In each experiment the pigs were divided into 6 replicated lots of 3 pigs each and allowed access to five self feeders containing the basal ration shown in table 1 but fortified with different antibiotics according to the following scheme: Feeder 1, no antibiotic; Feeder 2, 40 gm. chlortetracycline per ton; Feeder 3, 40 gm. oxytetracycline per ton; Feeder 4, 40 gm. penicillin V per ton; and Feeder 5, 40 gm. erythromycin per ton.

TABLE 1. COMPOSITION OF BASAL RATION FED IN EXPERIMENTS II AND III

Ingredients	Pounds
Ground yellow corn	78.5
Soybean oil meal	17.0
Vitamin premix [a]	2.0
Steamed bone meal	1.0
Ground limestone	0.9
Iodized salt	0.5
Trace mineral premix [b]	0.1
Total	100.0

[a] Choline chloride (25%), 160 gm.; Vitamin A (20,000 I.U./gm.), 10.0 gm.; Vitamin E (44 I.U./gm.), 9.1 gm.; Vitamin D_3 (9,000 I.U./gm.), 2.2 gm.; Niacin, 1.0 gm.; Vitamin B_{12} (0.5 mg./gm.), 0.5 gm.; Calcium pantothenate, 0.3 gm.; Thiamine, 0.1 gm.; Riboflavin, 0.09 gm.; Pyridoxine, 0.06 gm.; Folic acid, 0.05 gm.; and Soybean oil meal carrier, 724.6 gm.

[b] Manganous sulfate ($MnSO_4 \cdot H_2O$), 21.5%; Ferrous sulfate ($FeSO_4 \cdot H_2O$), 33.2%; Cupric sulfate ($CuSO_4 \cdot 5H_2O$), 1.8%; Cobaltous sulfate ($CoSO_4 \cdot H_2O$), 0.5%; Zinc sulfate ($ZnSO_4 \cdot H_2O$), 6.3%; Potassium sulfate (K_2SO_4), 1.6%; and Calcium carbonate carrier, 35.1%.

Feed consumption from each feeder was determined weekly and the feeders were shifted weekly as in Experiment I. Rations eaten so slowly as to become stale in the feeders were periodically replaced with fresh feed. The experiments were conducted in concrete pens and were of six weeks duration.

Results and Discussion

Results of the three experiments are summarized respectively in tables 2, 3 and 4.

In Experiment I (table 2) it was observed from the outset that the pigs avoided the protein supplement that contained erythromycin. The amount measured as having been consumed from this feeder was less than 1% of the total. It was also observed that the supplement containing chlortetracycline was most palatable. Almost half of the total consumption was eaten from the feeder containing this antibiotic. Penicillin V and oxytetracycline appeared to exert no particular influence on the pigs' preference. Both were

TABLE 2. THE PREFERENCE OF PIGS FOR VARIOUS ANTIBIOTICS WHEN OFFERED IN A PROTEIN SUPPLEMENT

Antibiotic	Supplement consumed	
	Pounds	Percent of total
Erythromycin	6.5	0.6
Oxytetracycline	221.0	20.4
Chlortetracycline	537.5	49.6
Penicillin V	319.0	29.4
Total	1084.0	100.0

consumed in significant quantities, with penicillin V being consumed as 29.4% of the total oxytetracycline as 20.4% of the total.

The feed consumption data of Experiment II (table 3) show a trend quite similar to the results of Experiment I. The differences are somewhat more striking in that there was an absolute refusal of the pigs to eat the ration containing erythromycin. All six pens of pigs showed a marked preference for the feed containing chlortetracycline, averaging two thirds of their total feed intake from this feeder. Again, oxytetracycline and penicillin V containing feeds were eaten in significant quantities but to a much lesser extent than that containing chlortetracycline. However, all three of these antibiotics appeared to enhance the palatability of the basal ration by virtue of the fact that less of the ration was consumed when no antibiotic was included. Some variation in preference was observed between pens, but in general the results were reasonably consistent from pen to pen.

As previously explained, Experiment III was a repeat of Experiment II except that the pigs used had not previously had access to an antibiotic supplemented feed. The results of Experiment III are presented in table 4. As in the previous experiment all six pens of pigs refused to eat any of the ration containing erythromycin. Their preference for chlortetracycline was

TABLE 3. RELATIVE PREFERENCE FOR CERTAIN ANTIBIOTICS BY WEANLING PIGS EXPRESSED IN TERMS OF PERCENT OF TOTAL FEED CONSUMPTION
(PIGS HAD BEEN FED CHLORTETRACYCLINE DURING THE SUCKLING PERIOD)

Antibiotic	Pen No.						Average, all pens
	1	2	3	4	5	6	
None	6.4	8.8	8.7	8.9	4.9	1.0	6.4
Chlortetracycline	66.9	77.8	71.6	74.0	50.4	56.8	66.2
Oxytetracycline	14.2	11.3	6.5	7.6	4.3	35.6	13.2
Penicillin V	12.6	2.1	13.2	9.5	40.4	6.6	14.2
Erythromycin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total feed consumption, lb.	438	522	462	462	508	481	479

again very pronounced and of approximately the same order of magnitude. However, there was a marked increase in the consumption of the basal ration containing no antibiotic at the expense of oxytetracycline and penicillin V. This may have been due to a conditioning response, since the pigs were accustomed to eating an antibiotic-free creep ration.

Statistical analysis of the data by the Duncan Multiple Range Test showed that the ration supplemented with chlortetracycline was significantly ($P < 0.01$) more palatable than any other rations. There were no other significant differences between the ration treatments.

It is difficult to explain the preferences shown by these pigs for the four antibiotics. Only crystalline antibiotics were used in this study, so the

TABLE 4. RELATIVE PREFERENCE FOR CERTAIN ANTIBIOTICS BY WEANLING PIGS EXPRESSED IN TERMS OF PERCENT OF TOTAL FEED CONSUMPTION (PIGS HAD NOT PREVIOUSLY BEEN FED ANY ANTIBIOTIC)

Antibiotic	Pen No. 1	2	3	4	5	6	Average, all pens
None	4.7	15.7	26.3	30.8	31.2	27.7	22.1
Chlortetracycline	86.5	77.8	56.9	61.6	44.4	53.6	64.9
Oxytetracycline	7.5	5.8	13.2	7.1	18.2	9.8	9.9
Penicillin V	1.3	0.7	3.7	0.5	6.2	8.9	3.2
Erythromycin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total feed consumption, lb.	600	585	547	594	455	440	539

differences should not be due to carrier materials or impurities. The only obvious points of difference lie in their molecular structures, and between chlortetracycline and oxytetracycline at least, this difference is small. These two have the tetracycline structure with five hydroxyl side groups in common, but chlortetracycline has a chloride ion attached to carbon 16, while oxytetracycline has an additional hydroxyl ion on carbon 12, Welch (1955). Penicillin V and erythromycin have little or no structural similarities to the tetracyclines. Assuming that molecular structure is responsible for these differences in palatability, the beneficial effect of chlortetracycline must be due almost entirely to its chloride ion.

Summary

Three experiments involving 62 pigs were conducted to determine the influence of chlortetracycline, oxytetracycline, penicillin V and erythromycin on the palatability of swine rations. Results obtained appear to justify the following statements.

Chlortetracycline enhanced the palatability of swine rations and was much preferred, when a choice was given, over oxytetracycline, penicillin V

and erythromycin. Oxytetracycline and penicillin V did not clearly improve or depress the acceptability of feeds containing them.

The results pertaining to erythromycin were very conclusive. Pigs completely avoided feed containing this antibiotic when other feed was available.

Since greater feed consumption is one of the sought-after goals in pork production, care must be exercised to prevent the fortification of growing-fattening swine rations with non-palatable materials. Since erythromycin appears to fall into this category it would seem logical to judge it as an unsuitable antibiotic for swine feeding purposes.

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EFFECT OF ADDING A CHOLAGOGUE TO RATIONS FOR GROWING SWINE AT THREE LEVELS OF DIETARY FAT [1]

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THE role of bile in lipid digestion and absorption is generally recognized. A number of agents have been shown to promote choleresis (Berman *et al.*, 1941); Finnegan and Emerson, 1953). Some choleretics increase bile volume without a corresponding increase in total solids. Others exhibit a "true" choleretic effect where the specific gravity is unchanged or increased as well as increasing volume output. Recently, Kleinberg (1955) demonstrated that the diethanolamine salt of the mono (+) camphoric acid ester of alpha, 4-dimethylbenzyl alcohol has a "true" choleretic effect when injected intravenously in dogs.

In view of the interest which has recently been shown in the addition of waste animal fats to animal rations the following experiment was conducted to study the effects of adding a choleretic to swine rations containing three levels of fat.

Experimental Procedure

Fifty-four weanling Duroc pigs were allotted to a 2 x 3 factorial experiment employing a completely randomized design. The main treatment effects were Gallogen [3] and fat.

Composition of the basal ration is presented in table 1. Gallogen was added at a level of 0.05% in all of the rations containing this supplement. Stabilized prime tallow was the source of fat which was added at levels of 0, 5, and 10% to the various rations. Where fat was added to the ration it replaced corn on an equal weight basis. Each ration was adjusted to contain approximately 18% crude protein.

All pigs were fed in concrete drylot with feed and water provided *ad libitum*. Individual weights were recorded at 2-week intervals and group feed consumption was obtained. Fecal samples were obtained per rectum from each pig at the end of four weeks on trial and again at the end of the eighth week to be used in determining fecal ether extract values.

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[2] Grateful acknowledgement is made to American Cyanamid Company, Pearl River, N. Y.; S. E. Massengill Company, Bristol, Tenn.; and Dawes Laboratories, Inc., Chicago, Ill. for supplying products used in this experiment. Acknowledgement is also due J. L. Carmon for assistance in the statistical evaluation of the results and to J. R. Danion for aid in caring for the animals.

[3] The diethanolamine salt of the mono (+) camphoric acid ester of alpha, 4-dimethylbenzyl alcohol manufactured by the S. E. Massengill Company, Bristol, Tenn.

TABLE 1. COMPOSITION OF BASAL RATION

Ingredients	Pounds
Ground yellow corn	71.2
Soybean oil meal	24.5
Dehydrated alfalfa meal	2.0
Limestone	0.5
Defluorinated phosphate	1.0
Salt	0.5
B-vitamin supplement[a]	0.1
Vitamin B_{12} supplement[b]	0.1
Antibiotic supplement[c]	0.1
Vitamins A and D[d]	+

[a] Calculated to provide the following quantities per lb. of feed: riboflavin, 2 mg.; pantothenic acid, 4 mg.; niacin, 9 mg.; choline, 10 mg.; and folic acid, 60 mcg.
[b] Calculated to provide 10 mcg. vitamin B_{12} per lb. of feed.
[c] Calculated to provide 10 mg. of chlortetracycline per lb. of feed.
[d] Calculated to provide 500 I.U. of vitamin A and 100 I.U. of vitamin D_3 per lb. of feed.

Results and Discussion

Average performance data for the various groups is presented in table 2. Analysis of covariance was used to adjust for differences in initial weights of the pigs in evaluating all growth data.

TABLE 2. SUMMARY OF AVERAGE PERFORMANCE DATA

	Levels of added fat, %			
	0	5	10	Averages
Basal ration				
No. of pigs	8[a]	9	7[a]	24
Av. initial wt., lb.	37.4	37.8	38.2	37.8
Av. final wt., lb.	169.1	178.4	187.9	178.5
Av. daily gain, lb.	1.57	1.67	1.78	1.67
Av. feed per lb. gain, lb.	3.30	3.05	2.70	3.02
Av. daily feed intake, lb.	5.24	5.10	4.72	5.06
Basal + Gallogen				
No. of pigs	9	9	9	27
Av. initial wt., lb.	41.0	38.9	39.1	39.7
Av. final wt., lb.	184.8	175.4	181.9	180.7
Av. daily gain, lb.	1.74	1.63	1.70	1.68
Av. feed per lb. gain, lb.	3.34	3.05	2.74	3.04
Av. daily feed intake, lb.	5.71	4.96	4.65	5.11
Averages				
No. of pigs	17	18	16	
Av. initial wt., lb.	39.2	38.3	38.7	
Av. final wt., lb.	176.9	176.9	184.9	
Av. daily gain, lb.	1.64	1.65	1.74	
Av. feed per lb. gain, lb.	3.33	3.05	2.72	
Av. daily feed intake, lb.	5.49	5.03	4.68	

[a] Pigs removed from these groups for reasons not associated with treatment. Values calculated by the missing plot technique were used.

A significant linear increase in rate of gain resulted from increasing the fat level of the ration. A significant Gallogen x fat linear interaction was also found. The response to the graded levels of fat in those rations not supplemented with Gallogen was a near perfect linear increase. However, those pigs receiving the rations containing the cholagogue did not exhibit this linear response. Instead, the cholagogue appeared to exert a negative regression effect on growth response. This regression effect appears to be at least partially explained on the basis of feed consumption. In the presence of the cholagogue average daily feed consumption was decreased on those rations containing added fat.

TABLE 3. PERCENTAGE ETHER EXTRACT AS DETERMINED FOR THE FEED AND FECES

Treatment	Ether extract of feed[a]	Ether extract of feces[a]
	%	%
	Period I	
Basal	3.71	7.62
5% added fat	7.99	8.98
10% added fat	12.86	12.19
Basal + Gallogen	3.27	6.02
5% added fat + Gallogen	8.22	5.23
10% added fat + Gallogen	12.87	15.00
	Period II	
Basal	4.72	7.31
5% added fat	8.81	10.46
10% added fat	12.47	12.16
Basal + Gallogen	4.67	7.95
5% added fat + Gallogen	8.71	7.85
10% added fat + Gallogen	13.28	9.56

[a] Dry matter basis.

Examination of the amount of feed required to produce a pound of gain (table 2) shows that in all instances there was an increase in the efficiency of feed utilization as the level of fat was increased in the ration. It may likewise be noted that the average daily feed intake was decreased as the level of fat was increased in the rations. No apparent differences in feed efficiency were obtained as a result of the addition of the cholagogue to the rations. However, the average daily feed intake and rate of gain was greater in the presence of the Gallogen on the ration without added fat, whereas there was a decrease in average daily feed intake on those rations containing both the cholagogue and added fat (table 2).

Ether extract was determined on each fecal sample and its corresponding feed sample at two different times during the course of the experiment. The averages of these values are reported in table 3. While it is recognized that this technique does not constitute an absolute measure of the ap-

parent absorption of the dietary fat intake, it was thought that it might give some indication of any effect the cholagogue might have in this regard.

There was considerable variation in the fecal ether extract values from pigs within treatment groups. This finding appears to be quite typical of fat excretion according to the reports of Hill and Bloor (1922) and Black and Fourman (1947). Statistical analysis of the ether extract values obtained from the fecal samples taken at the end of the first month indicated a highly significant increase in percentage of ether extract as the level of dietary fat was increased. Orthogonal comparisons revealed that 70% of the treatment variation was due to the fat linear component. There was no significant difference in percentage of fecal ether extract due to Gallogen as indicated by fecal samples obtained at the end of the first month.

Analysis of the fecal ether extract values obtained from the individual pigs at the end of the second month on experiment also revealed a highly significant increase in the ether extract fraction as the level of dietary fat was increased. A significant decrease in the percentage of fecal ether extract resulted from Gallogen supplementation. A significant Gallogen x fat linear interaction was found. Fecal lipid output appears to have been reduced in the presence of the cholagogue on rations containing the 5 and 10% added fat. Whether this represents an absolute increase in fat absorption cannot be definitely stated, since these values are not necessarily representative of total fecal output. However, the data tend to suggest an increase in fat absorption in the presence of the cholagogue. It is apparent from the data in table 3 that the linear increase in feed ether extract values with increasing fat level is largely found with the pigs receiving the rations not containing the cholagogue.

Backfat measurements were made by the live probe technique (Hazel and Kline, 1952) on all of the pigs at the termination of the experiment. When these data were subjected to analysis of covariance to adjust for differences in final weights of the pigs, a significant linear increase in backfat thickness was found as the level of fat in the ration was increased.

Summary

Fifty-four weanling pigs were used to study the effects of adding a choleretic agent to rations containing three levels of fat.

Increasing the fat level of the ration resulted in a significant increase in rate of gain. Addition of the cholagogue to the rations resulted in a negative regression effect on growth response as the fat level of the ration was increased.

Efficiency of feed utilization was increased in the order of increasing fat level in the ration, while average daily feed intakes decreased.

A linear increase in fecal ether extractable material was found as the level of dietary fat was increased. A significant decrease in the percentage of fecal ether extract resulted from supplementation with the cholagogue at the end of eight weeks on trial.

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REVALUATION OF THE L-LYSINE REQUIREMENT OF THE WEANLING PIG [1]

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MERTZ *et al.* (1949) demonstrated the dietary indispensibility of lysine for the growth of weanling pigs. Brinegar *et al.* (1950a) observed that when a ration containing 10.6% protein was fed to pigs, 0.6% L-lysine was needed for optimal growth. A lysine level of 1.2% was required, however, when the protein content of the diet was increased to about 22%.

Evidence was presented by Shelton *et al.* (1951a) indicating that the L-lysine requirement of weanling pigs fed a 23.8% protein purified diet was no higher than 1.0% of the ration, and possibly as low as 0.8%. The tentative recommended level of L-lysine for normal growth of weanling pigs which has been proposed by the National Research Council (1953) is 1.00% of the total diet.

More recently, however, several investigators have suggested that the minimum L-lysine requirement may be substantially lower than 1.00%. Henson *et al.* (1954) fed 30-lb. pigs a corn-tankage basal containing 14.5% protein and 0.63% L-lysine. Adding 0.6% DL-lysine did not improve either the growth rate or feed efficiency.

Becker *et al.* (1954a) found that pigs five to nine weeks old progressed satisfactorily on a diet containing 12% milk protein with a lysine level of 0.72% of the total ration. It was also reported (Becker *et al.*, 1954b) that for the 40 to 100 lb. pig, a 14% protein ration which sustained satisfactory performance contained only 0.63% lysine.

Meade (1956a) conducted nitrogen balances on 32 kg. pigs receiving a 15.9% crude protein diet with varying levels of L-lysine. The 0.69% lysine in the basal ration alone was adequate to promote acceptable nitrogen retention, which was not increased when supplemental lysine was given. In addition, satisfactory nitrogen retention occurred when growing pigs were fed a 14.2% protein diet containing 0.62% lysine (Meade and Teter, 1956). Normal gains were produced in pigs initially weighing 38 lb. when a 14.2% protein ration which contained 0.65% lysine was fed. Slightly reduced gains were recorded on a 13.4% protein diet containing 0.55% lysine (Meade, 1956b).

Hutchinson *et al.* (1956) reported that when 35 lb. pigs were fed either an 11.69 or 14.25% protein diet containing no more than 0.525%

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L-lysine, additional lysine caused no significant increase in growth rates.

The two experiments here reported were performed to specifically reinvestigate the L-lysine requirement of the weanling pig as determined by growth and feed efficiency data.

Experiment I

Experimental Procedure. The basal ration was an adaption of those used by Jackson *et al.* (1953), Beeson *et al.* (1953), and Mertz *et al.*

TABLE 1. COMPOSITION OF THE 0.40% L-LYSINE RATION, EXPERIMENT I

Ingredient	Percent of air-dry ration	Vitamin solution[e] Ingredient	Per 10 lb. pig wt.
Ground corn[a]	84.00	Calciferol	80.0 I.U.
Salt mix[b]	2.80	Mixed tocopherols	10.0 mg.
Trace mineral mix[c]	0.25	Menadione	0.8 mg.
Nine essential amino acids[d]	2.67	Ascorbic acid	20.0 mg.
L-lysine·HCl (95%)	0.32	Thiamine·HCl	1.0 mg.
Diammonium citrate	4.76	Riboflavin	1.6 mg.
Cerelose	4.70	Calcium pantothenate	6.0 mg.
Liver extract	0.50	Niacin	8.0 mg.
		Pyridoxine·HCl	0.8 mg.
	100.00	Choline·Cl	320.0 mg.
		B_{12} (crystalline)	10.0 mcg.
Vitamin A acetate	1,000 I.U./lb.	Biotin	80.0 mcg.
Delsterol	100 I.U./lb.	Folic acid	80.0 mcg.
Crystalline aureomycin·HCl	10 mg./lb.	Inositol	40.0 mg.
Vitamin solution[e]	Added daily	Para-aminobenzoic acid	40.0 mg.

[a] Contained 8.0% crude protein and 16.8% moisture by analysis (A.O.A.C., 1945).

[b] Composition in lb. per 100 lb. ration: $CaHPO_4 \cdot 2H_2O$ 1 lb., $CaCO_3$ 1 lb., NaCl (iodized) 0.5 lb., K_2HPO_4 0.3 lb. Total diet contained 0.65% Ca, 0.46% P, and 0.36% K.

[c] Provided the following amounts in parts per million of each element in the ration: Mg 310, Fe 84, Mn 43, Zn 53, Cu 8.4, F 5.3, Co 2.2.

[d] See table 2.

[e] Dissolved in a 30% ethyl alcohol solution in a concentration such that 1 ml. was given for each 10 lb. bodyweight per day.

(1954, 1955); that is, corn was supplemented with its deficient amino acids in purified form, and diammonium citrate was added to bring the total protein up to the desired amount. Six lysine levels were fed. The ration containing 0.40% total available L-lysine is given in table 1. The L-lysine HCl, diammonium citrate and cerelose levels were adjusted in the other five lots to provide 0.50, 0.60, 0.70, 0.80, and 1.00% available L-lysine in a ration containing a constant 12.9% total and 11.8% digestible crude protein equivalent.

The corn was assayed microbiologically for the ten essential amino acids plus tyrosine using Leuconostoc mesenteroides P-60 (Steele *et al.*, 1949). The analysis is given in table 2 with the amounts of the crystal-

line amino acids added to satisfy the lysine levels and the suggested requirements of the other amino acids as listed by the National Research Council. The DL-phenylalanine level fed was raised from 0.46% recommended by Mertz *et al.* (1954) to 0.50% for uniformity.

Eighteen Duroc barrow pigs, six weeks of age and averaging about 27 lb., were divided into six groups of three pigs each on the basis of weight and litter, and individually penned with wood shavings as bedding. The pigs were gradually adjusted to the experimental regimen during the three days immediately preceding the initiation of the experiment.

TABLE 2. AMINO ACID COMPONENTS OF THE BASAL RATION USED TO REVALUATE THE LYSINE REQUIREMENT OF THE WEANLING PIG EXPERIMENT I

Amino acid	Amino acid assay value of air-dry corn	Digestible amino acid in diet supplied by corn [a]	Added crystalline amino acid in diet	Form of crystalline amino acid added	Total utilizable amino acid [b]		
					In air-dry diet	In digestible crude protein of air-dry diet [c]	
	%	%	%		%	%	
Arginine	0.20	0.14	0.07	L·HCl	0.20	1.7	(1.5)
Histidine	0.18	0.13	0.10	L·HCl·H_2O	0.20	1.7	(1.5)
Isoleucine	0.29	0.20	1.00	DL	0.70	5.9	(5.4)
Leucine	0.82	0.58	0.02	L	0.60	5.1	(4.6)
Lysine	0.23	0.16	0.32–1.11	95% L·HCl	0.40–1.00	3.4–8.5	(3.1–7.7)
Methionine	0.18	0.13	0.47	DL	0.60	5.1	(4.6)
Phenylalanine	0.38	0.27	0.09	DL	0.36 } 0.50	3.0	(2.8)
Tyrosine	0.20	0.14	0.00		0.14 }	1.2	(1.1)
Threonine	0.27	0.19	0.42	DL	0.40	3.4	(3.1)
Tryptophan	0.04	0.03	0.17	DL	0.20	1.7	(1.5)
Valine	0.33	0.24	0.33	DL	0.40	3.4	(3.1)

[a] Assay value x percent of corn in diet x percent digestibility of corn protein (Atwater, W. O., 1902. Principles of nutrition and nutritive value of food. Farmers' Bulletin 142, U.S.D.A., pg. 26), or assay value x 84% x 84%.

[b] Digestible amino acid from corn plus physiologically active amino acid from the crystalline form.

[c] Digestible crude protein=11.8% of air-dry diet. Figures in parentheses denote percent of utilizable amino acid in total crude protein in the diet. Total crude protein=12.9% of air-dry diet.

Each pig was fed twice daily *ad libitum*, and on the evening feed the vitamin solution as given in table 1 was added from a pipette. Individual weight gains and feed consumption data were recorded weekly. The experiment was in progress for 28 days.

Results and Discussion. Table 3 contains a summary of the effects of the six lysine levels on growth, appetite and feed efficiency. Growth rate, feed consumption, and feed efficiency increased markedly as the lysine level was increased, with near maximum values attained by the 0.60% lysine lot.

The analysis of variance (Snedecor, 1946) showed that the 0.40% lysine lot gained significantly less ($P<0.01$) than the five higher lysine levels, and the 0.50% lysine group gained significantly less ($P<0.025$) than the combined 0.60, 0.70, 0.80 and 1.00% lysine lots. Similarly, the 0.40% lysine lot consumed significantly less feed ($P<0.025$) than the

groups receiving higher lysine levels, and the 0.50% lysine lot consumed significantly less ($P<0.05$) than the combined four lots receiving greater percentages of lysine. Among the 0.60, 0.70, 0.80 and 1.00% lysine groups, there were no significant differences in gain or feed consumption, indicating that the 0.60% lysine diet promoted growth and appetite as well as did any of the higher lysine levels.

Significant differences in feed efficiency were not found between lots but an obvious trend was established that, as the lysine level was increased from 0.40 to 0.60%, the feed required per pound of gain decreased. Protein efficiency, or pounds of gain produced for each pound of protein

TABLE 3. AVERAGE DATA FOR PIGS ON RATIONS CONTAINING SEVERAL LYSINE LEVELS, EXPERIMENT I [a]

Total utilizable L-lysine, air-dry, %	0.40	0.50	0.60	0.70	0.80 [b]	1.00
Initial weight, lb.	27.2	27.3	27.5	27.5	27.0	26.7
Final weight, lb.	43.3	49.3	64.5	56.3	66.3	64.5
Daily gain, lb. [c, d]	0.58	0.79	1.32	1.03	1.40	1.35
Daily feed consumption, lb. [c, e]	1.95	2.21	3.04	2.55	3.14	2.97
Feed per pound of gain, lb. [f]	3.38	2.81	2.30	2.47	2.24	2.20
Protein efficiency [g]	2.31	2.77	3.37	3.13	3.45	3.52

[a] Three barrow pigs per treatment. Duration of experiment, 28 days.
[b] One pig from this lot failed to respond. He was removed from experiment and his data discarded. Autopsy proved negative.
[c] Significant difference ($P<0.05$) between lots.
[d] Least significant difference 5% 0.47 lb.; 1% 0.66 lb.
[e] Least significant difference 5% 0.80 lb.; 1% 1.11 lb.
[f] No significant difference ($P>0.05$) between lots.
[g] Pounds gain per pound crude protein.

equivalent consumed, likewise tended to improve as the lysine level increased from 0.40 to 0.60%.

Apparently, 0.40% L-lysine was not enough to promote optimal results, and little if any advantage resulted from feeding a lysine level of over 0.60%.

Experiment II

Experimental Procedure. Because of the few replicates used per treatment in the first experiment, a second was undertaken in an attempt to confirm the results obtained. A fresh quantity of ground corn was procured and analyzed for total protein, moisture, and amino acids as before. The assay revealed a much lower moisture content than that used in Experiment I, 12.7% vs. 16.8%. In preparing the basal ration for Experiment II using this corn and making the ration as comparable as possible to that used in Experiment I, two procedures were considered. The first consisted in disregarding the inequality of the corns in moisture content and formulating the basal diet of Experiment II on an air-dry basis as was done in Experiment I. The objection to this approach was that 100 lb.

of the Experiment II ration would contain about four lb. less water, therefore be more concentrated in caloric value, but contain less of each supplemented nutrient per energy unit, than the first basal diet. The second method proposed that the rations fed in the two experiments be comparable on a dry-matter basis. This seemed to be preferable, since it had been shown by Hill and Dansky (1950, 1954) and Peterson *et al.* (1954) with chicks, Cowgill (1928) with adult dogs, and Sibbold *et al.* (1956) with weanling rats that the rate of feed consumption is primarily regulated by the energy content of the feed. Therefore, the latter technique was employed

TABLE 4. COMPOSITION OF THE BASAL RATIONS, EXPERIMENT II

	L-lysine (air-dry basis) %			
Ingredient	0.52	0.62	0.73	0.83
Ground corn [a]	83.35	83.35	83.35	83.35
Salt mix [b]	2.91	2.91	2.91	2.91
Trace mineral mix [c]	0.26	0.26	0.26	0.26
Nine essential amino acids [d]	2.60	2.60	2.60	2.60
L-lysine·HCl (95%)	0.48	0.61	0.75	0.89
Diammonium citrate	4.61	4.44	4.27	4.11
Cerelose	5.27	5.31	5.34	5.36
Liver extract	0.52	0.52	0.52	0.52
	100.00 [e]	100.00 [e]	100.00 [e]	100.00 [e]

[a] Contained 8.8% crude protein and 12.7% moisture by analysis (A.O.A.C., 1945).
[b] Same ratio between ingredients as in Experiment I. Total diet contained 0.68% Ca, 0.47% P, and 0.36% K.
[c] Provided the following amounts in parts per million of each element in the diet: Mg, 322; Fe, 87; Mn, 45; Zn, 55; Cu, 8.7; F, 5.5; Co, 2.3.
[d] See table 5.
[e] Vitamin A acetate, delsterol, crystalline aureomycin·HCl and vitamins were given identically to Experiment I.

and, although it introduced some confusion in expressing the lysine requirement, it is believed that this procedure, in providing the same amount of each nutrient per pound of dry feed between experiments, eliminated one source of variation which has commonly been overlooked, especially in investigations concerning animal requirements.

The omission of the 0.40 and 1.00% lysine test groups from Experiment II was indicated by results of the first trial. Consequently, only four levels of L-lysine were fed: 0.52, 0.62, 0.73 and 0.83%, air-dry basis. These corresponded to the 0.50, 0.60, 0.70 and 0.80% L-lysine levels of Experiment I. On a moisture-free or dry-matter basis, these figures are 0.58, 0.70, 0.82 and 0.93% L-lysine in both experiments.

The rations as fed are given in table 4. The diets were adjusted to provide a protein content equivalent to that furnished by Experiment I on a dry-matter basis, namely 15.0% total and 13.7% digestible crude

protein equivalent. (On an air-dry basis these figures are 13.4% and 12.2% respectively).

The corn was assayed microbiologically as before, and the analysis is presented in table 5 with the supplementary amounts of amino acids which were added to meet the essential amino acid needs adjusted for dry-matter.

Twenty Duroc pigs—twelve barrows and eight gilts, seven weeks of age, averaging about 24 lb.—were divided into four groups of five pigs each on

TABLE 5. AMINO ACID COMPONENTS OF THE BASAL RATION USED TO REVALUATE THE LYSINE REQUIREMENT OF THE WEANLING PIG, EXPERIMENT II

Amino acid	Amino acid assay value of air-dry corn	Digestible amino acid in diet supplied by corn [a]	Added crystalline amino acid in diet	Form of crystalline amino acid added	Total utilizable amino acid [b]		
					In air-dry diet	In digestible crude protein of air-dry diet [c]	
	%	%	%		%	%	
Arginine	0.30	0.21	0.00		0.21	1.7	(1.5)
Histidine	0.20	0.14	0.09	L·HCl·H_2O	0.21	1.7	(1.5)
Isoleucine	0.26	0.18	1.09	DL	0.73	5.9	(5.4)
Leucine	0.82	0.57	0.05	L	0.62	5.1	(4.6)
Lysine	0.23	0.16	0.48–0.89	95% L·HCl	0.52–0.83	4.3–6.8	(3.9–6.2)
Methionine	0.19	0.13	0.50	98% DL	0.62	5.1	(4.6)
Phenylalanine	0.34	0.24	0.05	DL	0.29 } 0.52	2.3	(2.2)
Tyrosine	0.33	0.23	0.00		0.23 }	1.9	(1.7)
Threonine	0.32	0.23	0.38	DL	0.42	3.4	(3.1)
Tryptophan	0.06	0.04	0.17	DL	0.21	1.7	(1.5)
Valine	0.40	0.28	0.27	DL	0.42	3.4	(3.1)

[a] Assay value x percent of corn in diet x percent digestibility of corn protein (Atwater, W. O., 1902. Principles of nutrition and nutritive value of food. Farmers' Bulletin 142, U.S.D.A., pg. 26), or assay value x 83.35% x 84%.

[b] Digestible amino acid from corn plus physiologically active amino acid from the crystalline form.

[c] Digestible crude protein=12.2% of air-dry diet. Figures in parentheses denote percent of utilizable amino acid in total crude protein in the diet. Total crude protein=13.4% of the air-dry diet.

the basis of weight, sex and litter. The animals were managed and fed in a manner similar to Experiment I. The trial was in progress for 35 days.

Results and Discussion. The results of this experiment as summarized in table 6 confirm the results of Experiment I. The lowest lysine level, 0.52%, reduced appetite with consequent lower daily gains and less efficient feed and protein utilization. The 0.62, 0.73 and 0.83% rations produced substantially identical results. Statistical examination of the data revealed a general lack of uniformity within treatments, the cause of which is discussed later. However, feed efficiency analysis revealed a significant difference between lots ($P<0.01$), with the 0.52% lysine lot significantly ($P<0.01$) less efficient than the three higher lysine levels.

Considering the large variation between pigs within treatments, it was interesting to observe a possible sex difference in the lysine requirement. A comparison of average daily gains between the sexes appears in table 7.

TABLE 6. AVERAGE DATA FOR PIGS ON RATIONS CONTAINING SEVERAL LYSINE LEVELS,[a] EXPERIMENT II

Total utilizable L-lysine, air-dry, %	0.52	0.62	0.73	0.83
Initial weight, lb.	23.8	23.8	23.7	23.8
Final weight, lb.	54.7	62.7	64.6	61.0
Daily gain, lb.[b]	0.88	1.11	1.17	1.06
Daily feed consumption, lb.[b]	2.47	2.76	2.78	2.60
Feed per pound of gain, lb.[c, d]	2.80	2.48	2.38	2.44
Protein efficiency[e]	2.65	3.00	3.14	3.04

[a] Three barrows, two gilts per treatment. Duration of experiment, 35 days.
[b] No significant difference ($P>0.05$) between lots.
[c] Highly significant difference ($P<0.01$) between lots.
[d] Least significant difference 5% 0.21; 1% 0.29.
[e] Pounds gain per pound protein.

The barrows responded identically to the barrows in Experiment I, with the break in growth rate falling between a lysine level of 0.5 and 0.6%. However, the females showed a uniform growth response with 0.5% lysine as effective as the higher levels. When this data is combined as in table 6, the differences noticeable in the males are reduced by the female effect, thereby contributing to the lack of significance in the pooled data. A statistical analysis performed on the combined growth data showed that the treatment by sex interaction was as large as the treatment effect alone, indicating a possible sex difference.

When the growth data of the barrows receiving comparable lysine levels in both experiments were combined and analyzed, it was found that there was a significant difference ($P<0.05$) between the average daily gains of the groups receiving the four lysine levels. Comparing the lowest L-lysine lot with the combined higher lysine levels revealed that the 0.58% L-lysine group gained significantly less ($P<0.01$) than the lots receiving 0.70% lysine or more. Table 8 contains a summary of the combined data with L-lysine values given on a dry-matter basis.

The finding that the lysine requirement of weanling pigs is no higher than 0.62% of the air-dry or 0.70% of the dry-matter of the ration confirms the work of Brinegar *et al.* (1950a) with the low protein diet and

TABLE 7. COMPARISON OF GROWTH AND FEED DATA BETWEEN SEXES OF PIGS ON RATIONS CONTAINING SEVERAL LYSINE LEVELS,[a] EXPERIMENT II

Total utilizable L-lysine, air-dry, %	0.52		0.62		0.73		0.83	
	Females	Males	Females	Males	Females	Males	Females	Males
Daily gain, lb.	1.04	0.78	0.97	1.21	1.19	1.16	0.90	1.17
Feed per day, lb.	2.87	2.20	2.45	2.96	2.95	2.67	2.29	2.80

[a] Two gilts, three barrows per treatment.

the more recent reports of adequate responses in swine with diets containing lysine levels between 0.60 and 0.70% (Henson *et al.*, 1954; Becker *et al.*, 1954b; Meade, 1956a, 1956b; Meade and Teter, 1956), but disagrees with Shelton *et al.* (1951a) and the high protein investigations of Brinegar *et al.* (1950a). It may be significant that the rations presently reported contained several ingredients not aded as such in either of the last mentioned diets. These included folic acid, vitamin B_{12}, biotin, inositol, para-aminobenzoic acid, ascorbic acid and antibiotic.

The lysine requirement as indicated above represents 4.7% of the total crude protein of the ration. The requirement for growing pigs as reported by other investigators cited ranges from 3.7 (Hutchinson *et al.*, 1956) to 6.0 (Becker *et al.*, 1954a), with an average value of 4.7% of the total protein.

The best average growth rates obtained by the pigs of Experiment II were substantially below the averages of Experiment I. These differences

TABLE 8. COMBINED AVERAGE DAILY GAIN DATA OF MALE PIGS FROM BOTH EXPERIMENTS I AND II [a]

Total utilizable L-lysine in dry matter, %	0.58	0.70	0.82	0.93
Average daily gain, lb.[b, *]	0.78	1.26	1.09	1.26

[a] Six pigs per treatment, except only five in 0.93% lysine lot.
[b] Least significant difference 5% 0.33 lb.; 1% 0.46 lb.
[*] Significant difference ($P<0.05$) between lots.

are considered to have been inherent in the experimental animals used. As will be noticed, the spring pigs used in Experiment I averaged about 3.4 lb. heavier at six weeks of age than the summer pigs of Experiment II at seven weeks.

Summary

Two experiments involving a total of 37 animals were conducted in an attempt to determine the L-lysine requirement of weanling pigs (about 25 lb.). A semi-purified diet consisting of over 80% ground corn, amino acids and other nutrients was fed containing six different L-lysine levels. Total crude protein equivalent of the diet was standardized at 15.0% on a moisture-free basis.

Under the conditions of the experiments, the level of utilizable L-lysine which supported maximum gains and feed efficiency for pigs six to seven weeks of age was 0.70% of the moisture-free ration (0.60% of a ration containing 14.1% moisture, or 0.62% of a 10.6% moisture ration), and is equivalent to 4.7% of the total crude protein, or 5.1% of the digestible crude protein of the ration.

This level of L-lysine supported average daily gains of over 1.0 lb.

per day, required less than 2.50 lb. feed per pound of gain, and produced 3.00 lb. or more gain per pound protein consumed.

Female pigs appear to require no more than 0.58% L-lysine in a moisture-free ration (0.52% of a 10.6% moisture diet) for satisfactory gains.

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AMBIENT AIR TEMPERATURE AND WEIGHT GAIN IN SWINE [1]

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WEIGHT increase or weight gain is used as one of the principal criteria for measuring experimental response. It is also a factor of prime importance in swine selection and swine production in general. That weight gain is affected by climatic factors is well known, and, as early as 1904, it was reported that air temperature was related to the magnitude of gain (Grisdale, 1904).

First attempts to relate weight gain of swine to air temperature over a range of temperatures and under controlled conditions were reported from this laboratory (Heitman and Hughes, 1949). This first report represented 4 trials, whereas the work reported herein is based on 24 (including the previously reported 4). The complete data have been analyzed to show the relationship of air temperature and liveweight to average daily gain.

Experimental

The swine in these trials have varied in weight from 60 to 450 lb. They were Berkshires, Durocs, Hampshires, Poland Chinas, and first generation crosses between several of these breeds. The experiments were carried on in the California Psychometric Chamber (Kelly *et al.*, 1948; Heitman, Kelly and Hughes, 1949; Bond *et al.*, 1952) under conditions previously described (Heitman and Hughes, 1949). The hogs were full-fed by hand all they would readily consume of standard balanced rations.

Hogs were usually studied in groups of four, but varying in size from two to five. The average experimental period was 7 days. During each period the air temperature was maintained constant. During all trials the air motion was relatively constant (25 feet per minute) and the relative humidity was held as near 50% as the equipment would allow. Relative humidity varied somewhat, however, especially at 40° F.

The data collected represent 24 different trials involving a total of 94 different hogs. Since hogs were weighed individually, the performance of each hog for each experimental period yields a measure of the relationship of average daily gain to constant air temperature and mean body weight. The total number of hog-periods, or data observations, was 367, and the distribution of these observations with the two independent variables is presented in table 1.

[1] This paper is one of a series from the cooperative project between the Agricultural Research Service, U. S. Department of Agriculture, and the Departments of Animal Husbandry and Agricultural Engineering of the University of California.

[2] Departments of Animal Husbandry and Agricultural Engineering, Davis, and Agricultural Research Service stationed at Davis, respectively.

TABLE 1. SIZE OF EXPERIMENTAL SAMPLES

(NUMBER OF OBSERVATIONS UNDER VARIOUS COMBINATIONS OF INDEPENDENT VARIABLES, X_2 AND X_3)

Mean liveweight, lb. (X_3)	Air temperature, °F. (X_2)								
	40°	50°	60°	70°	80°	90°	100°	110°	Total
Less than 125	1	7	13	51	13	11	8	7	111
125 to 175	4	7	8	15	4	2	9	2	51
175 to 225	4	8	5	18	14	14	13		76
225 to 275	5	6	8	14	2	17	11		63
275 to 325	2	2	2	8	3	5	3		25
More than 325	3	6	2	18	3	7	2		41
Total	19	36	38	124	39	56	46	9	367
Correlation Coefficient, $r_{X_1X_3}$[a]	−.33	+.36*	+.61**	[b]	−.57**	−.36**	−.53**	−.82**	

[a] X_1=average daily gain, lb. per pig.
[b] $r_{X_1X_3}$ (weights less than 180 lb.)=+.32**; $r_{X_1X_3}$ (weights greater than 180 lb.)=−.14.
* Statistically significant, $P<0.05$.
** Statistically highly significant, $P<0.01$.

Air temperature was grouped by 10° intervals between 35° and 115° F. Thus, for example, the 50° level included all observations from 45° to 55° F., and observations actually centered around 50° F. because of experimental design. Mean body weight of each hog was used as one variable. This was the mean weight during an experimental period. Since experimental periods were relatively short, the mean body weight and initial body weight differed relatively little even during periods of rapid growth.

Results

The correlation between average daily gain and mean body weight for each air temperature was calculated. The resulting correlation coefficients, r, are given in table 1. Significant positive correlation was demonstrated at 50° and 60° F., while significant negative correlation was obtained at 80°, 90°, 100° and 110° F. The regression line at 70° F. for daily gain

TABLE 2. EFFECT OF AMBIENT AIR TEMPERATURE AND MEAN LIVEWEIGHT ON RATE OF GAIN IN SWINE

AVERAGE DAILY GAIN, LB. PER PIG (X_1)

Mean liveweight, lb. (X_3)	Air temperature, °F. (X_2)							
	40°	50°	60°	70°	80°	90°	100°	110°
100		1.37	1.58	2.00	1.97	1.40	.39	−1.32
150	1.27	1.47	1.75	2.16	1.82	1.14	−.19	−2.60
200	1.19	1.57	1.91	2.22	1.67	.88	−.77	
250	1.10	1.67	2.08	2.14	1.51	.62	−1.36	
300	1.02	1.77	2.24	2.06	1.36	.36	−1.95	
350	.94	1.87	2.41	1.98	1.21	.10	−2.53	

on body weight is not a straight line because heavy pigs gained at a maximum below 70°, whereas the lighter weight pigs gained most rapidly above 70° F. The data fit two straight lines joining at 180 lb. body weight. At weights less than 180 lb., there is significant positive correlation, while at heavier weights correlation is negative and lacks statistical significance. The correlation at 40° F. was negative and lacked statistical significance.

The data were analyzed as a joint function for two independent variables by the method of Ezekiel (1945). Values were smoothed through three approximation curves, twice with reference to body weight (X_3) and once with reference to air temperature (X_2).

Probable values of average daily gain (X_1) for specific combinations of air temperature (X_2) and body weight (X_3) may be seen in the correlation surface shown in three dimensions in figure 1. The warping of this

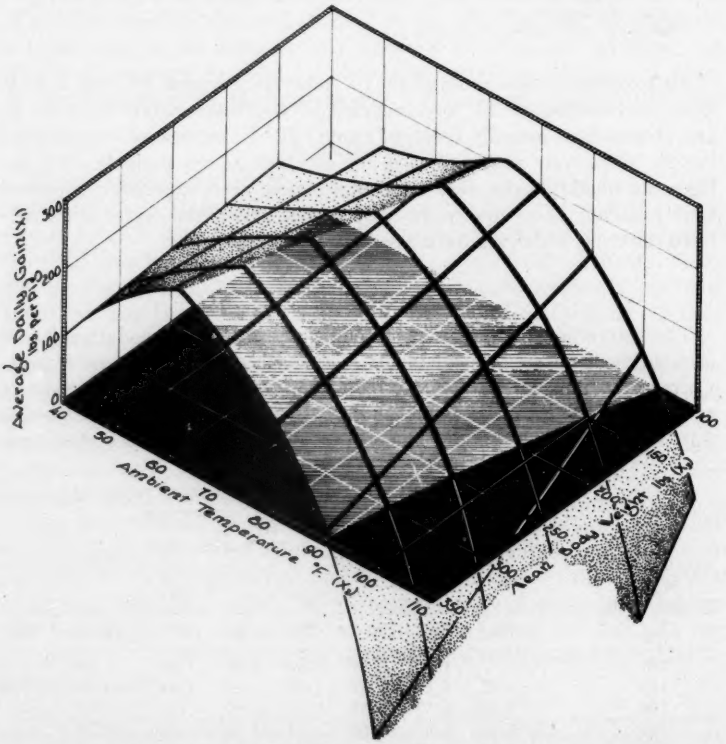

Figure 1. Probable values of average daily gain (X_1) for specific combinations of ambient air temperature (X_2) and mean body weight (X_3).

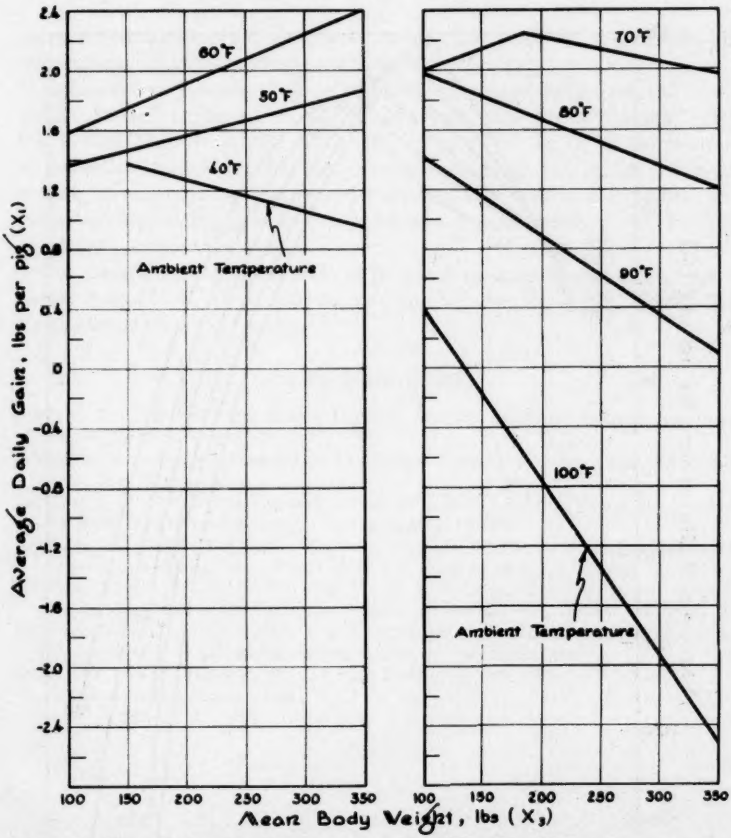

Figure 2. The relationship between average daily gain (X_1) and mean body weight (X_3) at various ambient air temperatures (X_2).

surface reflects the change in sign of the correlation coefficients given in table 1.

The probable values of average daily gain are plotted against body weight for the various air temperatures in figure 2 and against air temperature for various body weights in figure 3. Figures 1, 2, and 3 are based on smooth values as described above. These smoothed data are tabulated in table 2.

Summary

Data are reported from 24 experiments involving 94 hogs and 367 hog-periods. A three-dimension correlation surface was developed showing probable values of average daily gain for specific combinations of constant

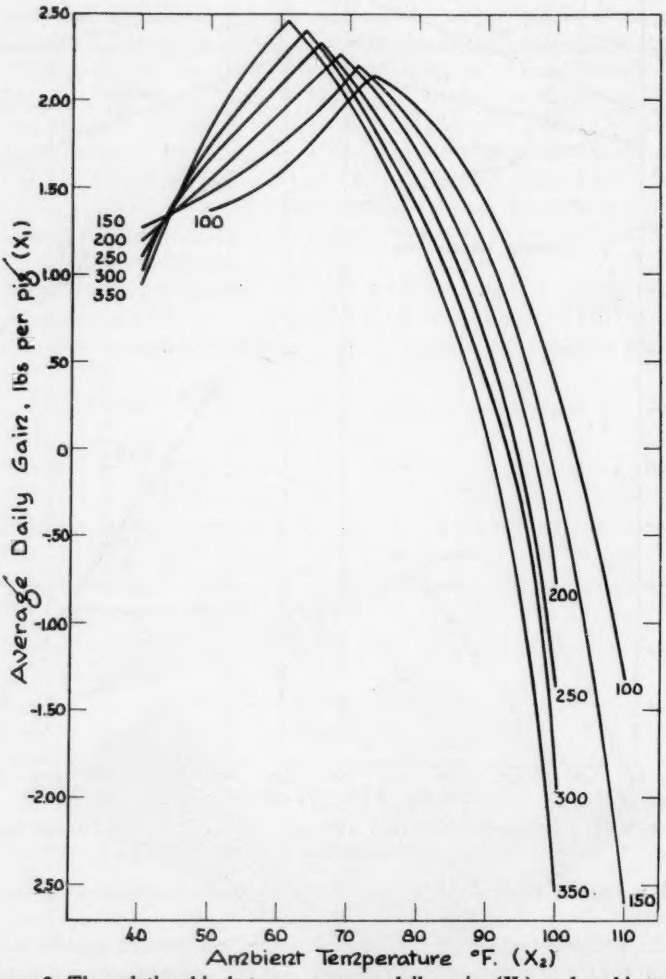

Figure 3. The relationship between average daily gain (X_1) and ambient air temperature (X_2) at various mean body weights (X_3).

air temperature (40° to 110° F.) and body weight (100 to 350 lb.). This surface was smoothed through three approximation curves as a joint function of two independent variables.

Probable value curves for average daily gain are also plotted against

body weight for constant air temperature at 10° intervals and against air temperature for various body weights at 50-lb. intervals.

Significant correlation coefficients between average daily gain and body weight for air temperatures at 10° intervals were positive at 50° and 60° F. and negative at 80°, 90°, 100° and 110° F. At 70° F. correlation at weights less than 180 lb. was positive and significant, while at higher weights it was negative and lacked statistical significance. Correlation between average daily gain and body weight was negative at 40° F., but also lacked significance.

The constant air temperatures at which daily gain was at a maximum varied from 61° F. for 350-lb. hogs to 73.5° F. for 100-lb. pigs under the conditions of these experiments.

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A STUDY OF XANTHURENIC ACID EXCRETION BY SWINE[1]

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NUTRITIONAL interest in xanthurenic acid originated with its isolation by Lepkovsky and coworkers (1943) from the urine of pyridoxine-deficient rats, and its concentration in urine has been widely used as an index of the state of vitamin B_6 nutrition. The urine of higher animals contains variable quantities of xanthurenic acid as a normal metabolite of tryptophan. The excretion rate is elevated in vitamin B_6 deficiency, in diabetes (Kotake and Tani, 1953) and in some normal animals after a test load of tryptophan (Brown and Price, 1956). Reports that women in middle and late pregnancy excrete increased amounts of xanthurenic acid following tryptophan administration (Wachstein and Gudaitis, 1952a), and that pyridoxine may be of value in treating nausea of pregnancy, have created an interest in the effect of gestation on vitamin B_6 metabolism and requirements.

Cartwright and associates (1944) have reported that the urinary excretion of xanthurenic acid is increased in pyridoxine deficient pigs. The present study was designed to determine the pattern of excretion of xanthurenic acid by non-pregnant and pregnant sows and growing pigs, and the effect of pyridoxine, tryptophan and insulin administration.

Experimental

Sows and gilts from the University breeding herd were placed in individual collection cages and urine was collected in glass bottles for four consecutive 24-hour periods. Urine obtained during the first period was discarded and that of the second period was used for estimating the basal excretion of xanthurenic acid. Treatments were introduced at the end of the second period and their effect was estimated from the excretion of xanthurenic acid during the next 24 or 48 hours.

The ration given the animals prior to and during the observation periods was of the following composition (%): ground yellow corn 82.6, soybean meal 12.2, dehydrated alfalfa meal 2.3, ground limestone 1.0, steamed bone meal 1.0, trace mineralized salt 0.5. Riboflavin, calcium pantothenate, niacin and choline chloride were added at a rate of 1, 2, 4.5 and 45 mg. per lb., respectively, and 440 I.U. of vitamin A, 82 International Chick Units of Vitamin D_3, and 5 mg. chlortetracycline were provided per lb. of feed. Each animal was given 6 lb. of ration at the beginning of each 24-hour collection period. Pyridoxine hydrochloride or DL-tryptophan

[1] The authors gratefully acknowledge the gift of DL-tryptophan and a sample of xanthurenic acid from Merck-Sharp & Dohme, Inc., Rahway, N. J., and of vitamins and Aureomycin from American Cyanamid Company, Pearl River, N. Y.

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supplements were administered by mixing uniformly with the daily ration.

Estimation of xanthurenic acid is based on its reaction with ferric salts to form a green color. Three methods were examined for their suitability when applied to swine urine. The procedure of Miller and Baumann (1945), involving extraction of xanthurenic acid in alcohol-ether, was found to be tedious for routine application. The method of Rosen and associates (1951) gave variable values and often low recoveries, which appeared to be associated with the high concentration of urinary pigments in some samples and with the difficulty of standardizing all operations in the procedure. Simplification of this method by Wachstein and Gudaitis

TABLE 1. XANTHURENIC ACID EXCRETION OF NON-PREGNANT GILTS AS AFFECTED BY TRYPTOPHAN AND/OR PYRIDOXINE ADMINISTRATION

Animal no.	Body wt. (kg.)	Xanthurenic acid (XA) excretion per 24-hr. period (mg.)					
		Period 1		Period 2		Period 3	
		Treatment	XA	Treatment	XA	Treatment	XA
1	104	none	34	T[a]	265	none	62
2	111	"	63	"	246	"	89
3	107	"	56	"	357	"	144
4	104	"	50	"	227	"	183
2	111	"	83	B_6[b]	50	B_6+T[a]	244
4	104	"	62	"	50	"	260
5	125	"	105	B_6+T[c]	270	B_6+T[c]	405
6	114	"	180	"	259	"	372
2	111	"	92	B_6	69	B_6	65
4	104	"	19	"	58	"	71
7	132	"	71	"	29	..	...

[a] DL-tryptophan, 21 gm.
[b] Pyridoxine hydrochloride, 25 mg., used throughout.
[c] DL-tryptophan, 12 gm. All supplements were mixed with the daily ration.

(1952b), in which the samples are buffered with tris (hydroxymethyl) amino methane prior to color development, gave reproducible values and recoveries of 95–105%. In reading a series of samples in an Evelyn photoelectric colorimeter, it was found convenient to determine an air blank reading on each tube, then develop the color and reread after adjusting the galvanometer to the proper air blank value. A determination was thereby made on each tube.

Results and Discussion

The xanthurenic acid excretion of non-pregnant gilts fed the basal ration alone or with supplements of pyridoxine hydrochloride and/or DL-tryptophan is indicated in table 1. As reported for man and other species, considerable variation was observed in the xanthurenic acid excretion of different animals and of the same animal at different periods. The increase in excretion following tryptophan administration is evident,

however, no modification of the tryptophan effect was obtained by simultaneous administration of pyridoxine. There is no indication, moreover, that the daily excretion of xanthurenic acid was reduced by administration of pyridoxine alone.

Corresponding data for pregnant gilts and sows are given in table 2. It is clear that the administration of pyridoxine resulted in no reduction in xanthurenic acid excretion. The apparent increase in period 3 was statistically insignificant ($P=>.05$). The sows in advanced pregnancy which were given a tryptophan load exhibited a characteristic increase in xanthurenic acid excretion, and the increase was of the same order as that observed in non-pregnant animals.

TABLE 2. XANTHURENIC ACID EXCRETION BY PREGNANT GILTS AND SOWS AS AFFECTED BY TRYPTOPHAN OR PYRIDOXINE ADMINISTRATION

			Xanthurenic acid (XA) excreted per 24-hr. period (mg.)					
			Period 1		Period 2		Period 3	
Animal no.	Body wt. (kg.)	Weeks pregnant	Treatment	XA	Treatment	XA	Treatment	XA
1	166	4	none	100	B_6[a]	113	B_6	187
2	120	4	"	83	"	82	"	120
3	182	6	"	160	"	149	"	117
4	161	6	"	63	"	58	"	82
5	179	12	"	106	"	72	"	81
6	177	13	"	57	"	133	"	142
7	184	14	"	70	"	81	"	132
8	184	14	"	81	"	191	"	149
9	200	14	"	100	T[b]	322	none	240
10	182	15	"	164	"	222	"	81
11	182	15	"	54	"	426	"	246

[a] Pyridoxine hydrochloride, 25 mg.
[b] DL-tryptophan, 25 gm.

Evidence has been presented from several sources that the pig exhibits a relatively low tolerance for glucose. Link (1953) has indicated that pigs are susceptible to carbohydrate-induced diabetes. The observations of Kotake (1953) on the xanthurenic acid excretion of diabetics, and the magnitude of the excretion observed in this study, suggested the possibility that insulin might induce a decrease in the excretory level. Accordingly, 25 units of protamine zince insulin plus 25 units of zinc-insulin crystals (Lilly) were administered simultaneously by intramuscular injection to a group of gilts. The data on xanthurenic acid excretion, shown in table 3, reveal no consistent effect of insulin administration on the excretory level of this pigment. Insulin also failed to modify the increased level of excretion following a DL-tryptophan load. The presence of relatively large amounts of xanthurenic acid in the urine of the pig is therefore apparently unrelated to the tolerance of this animal for glucose.

TABLE 3. EFFECT OF INSULIN ADMINISTRATION ON THE XANTHURENIC ACID EXCRETION OF NON-PREGNANT GILTS

Animal no.	Body wt. (kg.)	Xanthurenic acid (XA) excretion per 24-hr. period (mg.)					
		Period 1		Period 2		Period 3	
		Treatment	XA	Treatment	XA	Treatment	XA
1	104	none	47	I[a]	10	I+T[c]	354
2	107	"	62	"	49	"	103
3	123	"	105	B_6+T[b]	225	I+T[d]	231
4	138	"	58	"	156	"	144
1	104	"	11	I	58	I	82
5	107	"	36	"	63	"	29
6	113	"	115	"	61	..	...

[a] Protamine zinc insulin, 25 units, plus 25 units zinc-insulin crystals (Lilly) injected intramuscularly.
[b] Pyridoxine hydrochloride, 25 mg., and 12 gm. DL-trytophan mixed with the ration.
[c] DL-tryptophan, 21 gm.
[d] DL-tryptophan, 12 gm.

For purposes of comparison the excretion of xanthurenic acid was also determined for a group of young pigs, averaging 12.7 kg. in weight, which were growing actively on a purified dry diet. The diet provided 22.5% protein and was composed of 20% Labco casein, 30% dried whey (12.7% protein), 40% Cerelose, 3% corn oil, 3% woodflock, plus vitamins and minerals. Pyridoxine hydrochloride was present in the diet at a concentration of 4 mcg. per gram. Ten consecutive 12-hour urine collections were made on seven animals. The daily excretion of xanthurenic acid for the 5 days is indicated in table 4. There is evidence of the expected association between the rate of excretion of this compound and food consumption. The over-all excretory level of these young pigs is of the same general order as that of the gilts and sows when differences in food consumption are considered.

TABLE 4. XANTHURENIC ACID EXCRETION OF GROWING PIGS FED A BALANCED PURIFIED DIET

(MG./24 HOURS)

Pig no.	Body wt. (kg.)	Diet consumed daily (kg.)	Day number					Mean	$S_{\bar{x}}$
			1	2	3	4	5		
1	14.1	.90	6.7	6.5	14.0	12.4	16.4	11.20	1.98
2	11.4	.60	8.1	15.6	12.1	5.8	7.0	9.72	1.81
3	14.1	.90	15.2	22.5	16.6	7.8	16.0	15.20	2.39
4	11.3	.70	10.0	11.2	8.3	11.6	10.2	10.26	0.57
5	10.4	.60	12.5	8.0	8.9	12.2	7.3	9.78	1.08
6	13.2	.60	....	11.6	20.9	15.2	14.0	15.43	1.97
7	14.5	.90	16.3	31.1	14.5	10.7	8.4	16.20	3.97
Mean	12.7	.74	11.13	15.21	13.61	10.81	11.33		
$S_{\bar{x}}$[a]	.62	.058	1.45	3.31	1.66	1.18	1.54		

[a] Standard error.

On the basis of the urinary excretion of xanthurenic acid, no evidence of a disturbance of tryptophan or vitamin B_6 metabolism in pregnant gilts or sows was found in this study. The excretion level rose sharply following tryptophan administration, however, and xanthurenic acid was found in significant quantities in the urine. Vandelli and coworkers (1955) have reported that no increase in the excretion of this pigment occurred after administration of tryptophan (100 mg./kg.) to pregnant rats, rabbits, guinea pigs or dogs, in contrast to pregnant women. Brown and Price (1956) have found that man and the non-pregnant rat, but not the cat and dog, exhibit an increased xanthurenic acid excretion following a tryptophan load.

Summary

A study is reported of the urinary xanthurenic acid excretion of pregnant and non-pregnant gilts and sows, and of growing pigs. No evidence was found of a derangement of tryptophan or pyridoxine metabolism associated with pregnancy, such as that reported for some human subjects. Administration of DL-tryptophan resulted in an elevation of xanthurenic acid excretion which was unaffected by simultaneous administration of pyridoxine. Insulin had no effect on the excretion of xanthurenic acid, indicating that the substantial amounts of this compound found in the urine are not associated with the poor glucose tolerance which is alleged to be characteristic of the species.

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AVAILABILITY OF THE PHOSPHORUS FROM VARIOUS PHOSPHATE MATERIALS FOR SWINE [1]

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MANY supplements are being used as sources of phosphorus for swine. Some of these products have become available only recently. Many of the other more established products have been changed in recent years by changes in production methods. The comparable values of these products for poultry have been established by recent research but similar data for swine are meager and conflicting. Gillis *et al.* (1954) made a detailed study of the biological values of several inorganic phosphates for the chick, utilizing beef blood fibrin as the source of protein in the very low phosphorus basal rations. The biological values expressed as percent of the value of 100 assigned beta tricalcium phosphate were: dicalcium phosphate, 98; steamed bone meal, 70 to 100 depending upon the source; defluorinated phosphates, 82 to 99; Curacao Island phosphate, 87; and soft phosphate with colloidal clay less than 50. Several recent studies have indicated that the phosphorus in soft phosphate with colloidal clay is poorly available to farm animals and poultry. Such results were reported for the chick by Grau and Zweigart (1953), Johnson and Phillips (1953), Miller and Joukovsky (1953), and Motzok and Branion (1956); for turkey poults by Wilcox *et al.* (1954); for cattle by Long *et al.* (1956); and swine by Chapman *et al.* (1955). On the other hand, Gobble *et al.* (1956) reported a series of experiments indicating that the phosphorus of dicalcium phosphate and soft phosphate with colloidal clay were equal in biological availability to growing fattening swine. It appears from the studies to be reported here that the phosphorus content of the basal rations used by Chapman *et al.* (1955) and especially by Gobble *et al.* (1956) were not low enough in phosphorus to permit a critical evaluation of the supplements tested. In studies reported by Menzies *et al.* (1955), phosphoric acid phosphorus was at least as available as the phosphorus in steamed bone meal to beef heifers.

The purpose of this study was to compare steamed bone meal, defluorinated phosphate, Curacao Island phosphate, monocalcium phosphate, phosphoric acid, dicalcium phosphate and soft phosphate with colloidal clay as sources of phosphorus for growing swine. U.S.P. grade dicalcium phosphate was the reference source in all trials. Table 1 shows the

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[2] Contribution from the Department of Animal Husbandry, Journal Paper No. 1139, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

calcium, phosphorus and fluorine content of the composite samples of each product.

Experimental Procedure

Semi-purified diets were used in the first five experiments and a corn-soy diet was used in the sixth experiment. Duroc pigs weaned at either 6 or 8 weeks of age were used in all experiments. The criteria used in evaluating the supplements were growth rate, feed consumption, feed efficiency, general appearance, blood serum inorganic phosphorus and femur analyses. The femurs were analyzed for ash, fluorine, phosphorus and breaking strength. Also, in Experiment I observations were made on the appearance of the teeth at slaughter

TABLE 1. COMPOSITION OF THE PHOSPHORUS SUPPLEMENTS[a]

Supplement	Ca	P	F
	%	%	%
Dicalcium phosphate, precipitated, U.S.P.	23.9	18.6	0.0
Steamed bone meal	27.1	13.1	0.0
Defluorinated phosphate	34.7	13.6	0.07
Monocalcium phosphate, commercial grade	15.5	22.9	0.04
Curacao Island phosphate	34.8	15.2	0.5
Phosphoric acid, reagent grade[b]	0.0	23.7	0.0
Soft phosphate with colloidal clay	18.1	9.2	1.51

[a] Analyses by Calcium Carbonate Company, Quincy, Illinois.
[b] The concentrated phosphoric acid (85%) was diluted to 75% with distilled water before mixing in rations.

In the semi-purified diet studies six pigs were fed individually *ad libitum* per treatment. Each animal was quartered in a 3.5 by 7 foot concrete floored indoor pen and was bedded with ground corn cobs. Blood serum inorganic phosphorus determinations were made on each pig at the beginning of the feeding period and every 2 weeks thereafter (Fiske and Subbarow, 1925). Feed and weight records were taken every 7 days and detailed visual observations were made of each pig at that time.

Tables 2, 3 and 4 show the basal rations used in the semi-purified diet studies. Commercial casein was used as the source of protein in Experiments I and II and beef blood fibrin was used in Experiments III, IV, and V. Before adding the phosphorus supplements the casein basal contained 0.18% phosphorus and the blood fibrin basal 0.035% phosphorus. Thus the latter permitted a more critical evaluation of the phosphorus supplement. One-half of the cerelose was replaced with corn starch in Experiments III, IV and V to make the rations less laxative and less dusty. Even though no incidence of parakeratosis was ever noted in any animal, other research indicated the calcium level was higher than needed in Experiments I, II, and III and was thus lowered in Experiments IV and V. All rations

TABLE 2. COMPOSITION OF THE SEMI-PURIFIED CONTROL RATIONS

Ingredient	Experiment number				
	I	II	III	IV	V
	%	%	%	%	%
Cerelose (glucose)	65.12	69.40	34.17	34.63	34.418
Corn starch, globe	0.0	0.0	35.00	35.00	35.00
Casein, commercial	19.00	19.00	0.0	0.0	0.0
Beef blood fibrin	0.0	0.0	19.00	19.00	19.00
Alfalfa meal, 17%	5.00	0.0	0.0	0.0	0.0
Cellufloor (ground cellulose)	3.00	4.00	4.00	4.00	4.00
Corn oil	3.00	3.00	3.00	3.00	3.00
Liver meal	0.50	0.50	0.50	0.50	0.50
Calcium carbonate	1.40	1.40	0.98	0.52	0.617
Dicalcium phosphate	0.67	0.67	1.32	1.32	1.435
Mineral mixture [a]	1.21	1.21	1.21	1.21	1.21
Vitamin mixture [b]	0.50	0.22	0.22	0.22	0.22
Chlortetracycline supp.[c]	0.40	0.40	0.40	0.40	0.40
Vitamin A and D oil [d]	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00
Total calcium, percent	0.90	0.90	0.82	0.65	0.65
Total phosphorus, percent	0.30	0.30	0.275	0.275	0.30
Percent P supplied by supp.	0.12	0.12	0.24	0.24	0.265

[a] See table 3.
[b] See table 4.
[c] Added to supply 7.0 mg. per pound of ration.
[d] Contained 3,000 I.U. of vitamin A and 400 I.U. of vitamin D per gram.

TABLE 3. COMPOSITION OF THE MINERAL MIXTURES USED IN THE SEMI-PURIFIED BASAL RATIONS

Ingredients [a]	Experiment		
	I	II	III, IV & V
	lb.	lb.	lb.
Iodized salt	46.200	50.081	49.825
Magnesium carbonate ($MgCO_3$)$_4$Mg(OH)$_2$5H_2O	19.640	19.635	19.635
Potassium chloride (K Cl)	28.640	28.644	28.644
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	4.850	0.970	0.970
Cupric sulfate ($CuSO_4$)	0.067	0.067	0.067
Zinc oxide (Zn O)	0.037	0.037	0.295
Cobalt carbonate (Co CO_3)	0.037	0.037	0.037
Manganese sulfate ($MnSO_4 \cdot H_2O$)	0.527	0.527	0.527
Total	100.00	100.00	100.00

[a] All minerals except the iodized salt were U.S.P. grade or equal.

TABLE 4. COMPOSITION OF THE VITAMIN MIXTURE UESD IN THE SEMI-PURIFIED BASAL RATIONS

Ingredient	Experiment		
	I	II	III, IV & V
	gm.	gm.[a]	gm.[a]
Thiamine	0.5	0.7	0.7
Riboflavin	1.2	1.2	1.2
Niacin	8.0	8.0	8.0
Calcium pantothenate	6.0	6.0	6.0
Pyridoxine	0.6	0.8	0.8
Choline chloride	400.0	200.0	200.0
2 methyl napthoquinone	2.0	2.0	2.0
Folic acid	0.3	0.3	0.3
Mixed tocopherols	100.0	10.0	0.0
Alpha tocopherol	0.0	0.0	10.0
Vitamin B_{12} supp. (10 mg./lb.)	454.0	454.0	454.0
Casein, commercial	527.4	317.0	0.0
Beef blood fibrin	0.0	0.0	317.0
Total	1500.0	1000.0	1000.0

[a] These figures also equal the mg. per pound of total ration in Experiments II, III, IV, and V.

were calculated to be adequate in all required nutrients except phosphorus as recommended by the National Research Council (1953).

The several phosphorus supplements were added to the basal low phosphorus rations in amounts to supply the levels of total phosphorus shown in table 6. The right femurs from each pig in Experiments I, II, III and IV

TABLE 5. COMPOSITION OF THE RATIONS USED IN EXPERIMENT VI

Ingredient	Lot 1	Lot 2	Lot 3
	%	%	%
Ground yellow corn	74.375	74.044	73.742
Soybean oil meal	21.0	21.0	21.0
Alfalfa meal, 17%	2.5	2.5	2.5
Antibiotic suppl. (10 gm./lb.)[a]	0.075	0.075	0.075
B-vitamin supplement[b]	0.1	0.1	0.1
B_{12} supplement[c]	0.1	0.1	0.1
Salt, iodized	0.5	0.5	0.5
Calcium carbonate	1.35	0.875	1.35
Dicalcium phosphate	0.0	0.806	0.0
Phosphoric acid (reagent 75%)	0.0	0.0	0.633
Calcium, percent	0.65	0.65	0.65
Phosphorus, percent	0.30	0.45	0.45
Protein, percent	16.0	16.0	16.0

[a] Chlortetracycline.

[b] The B-vitamin supplement contained 2,000 mg. riboflavin, 4,000 mg. pantothenic acid, 9,000 mg. niacin and 8,679 mg. choline chloride per pound.

[c] The B_{12} supplement contained 9+ mg. of vitamin B_{12} per pound.

were analyzed for ash, phosphorus and fluorine. The ash and phosphorus analyses were made according to A.O.A.C. methods (1955). Fluorine determinations on the feed and bones were made by a commercial labora-

TABLE 6. SUMMARY OF GROWTH AND FEED DATA*

Lot	Treatment	Av. initial weight	Av. final weight	Phosphorus in ration			Av. daily gain	Av. daily feed	Feed per lb. gain
				Basal	Added	Total			
		lb.	lb.	%	%	%	lb.	lb.	lb.
				Experiment I—90 days					
1	Di-Cal	41.3	202.1	0.18	0.12	0.30	1.79	5.64	3.15
2	Soft phos.	41.2	198.5	0.18	0.12	0.30	1.75	5.90	3.37
3	Di-Cal	40.6	209.9	0.18	0.27	0.45	1.88	6.50	3.46
4	Soft phos.	40.7	210.6	0.18	0.27	0.45	1.79	5.75	3.21
5	Di-Cal	40.3	196.7	0.18	0.42	0.60	1.74	5.69	3.28
6	Soft phos.	40.6	181.8	0.18	0.42	0.60	1.68	5.20	3.31
				Experiment II—63 days					
1	Di-Cal	39.2	124.0	0.18	0.12	0.30	1.35	4.20	3.12
2	Bone meal	39.1	132.6	0.18	0.12	0.30	1.42	4.08	2.88
3	Defluor. phos.	39.3	121.7	0.18	0.12	0.30	1.31	3.98	3.04
4	Mono-Cal	39.0	128.3	0.18	0.12	0.30	1.42	4.04	2.85
5	Curacao	39.7	131.6	0.18	0.12	0.30	1.46	4.29	2.94
6	Soft phos.	39.4	124.7	0.18	0.12	0.30	1.35	3.85	2.84
				Experiment III—56 days					
1	Di-Cal	35.3	114.0	0.035	0.24	0.275	1.41	3.74	2.66
2	Mono-Cal	35.3	109.6	0.035	0.24	0.275	1.33	3.55	2.67
3	Soft phos.	35.7	99.8	0.035	0.24	0.275	1.15*	3.29	2.87
				Experiment IV—56 days					
1	Di-Cal	25.9	103.4	0.035	0.24	0.275	1.38	3.16	2.28
2	Mono-Cal	25.8	102.1	0.035	0.24	0.275	1.36	3.11	2.28
3	Bone meal	25.9	102.4	0.035	0.24	0.275	1.37	3.21	2.35
4	Curacao	25.8	95.0	0.035	0.24	0.275	1.24	2.70	2.18
5	Soft phos.	25.9	70.6	0.035	0.24	0.275	0.84**	2.28**	2.72**
6	Di-Cal+CaF_2	25.8	106.2	0.035	0.24	0.275	1.44	3.11	2.16
				Experiment V—70 days					
1	Di-Cal	19.8	90.7	0.035	0.265	0.30	1.02	2.54	2.51
2	H_3PO_4	20.0	96.6	0.035	0.265	0.30	1.10	2.82	2.57
				Experiment VI—107 days					
1	Corn-soy basal	32.4	171.2	0.30	0.00	0.30	1.30	4.95	3.8
2	Basal+Di-Cal	32.2	197.0	0.30	0.15	0.45	1.53**	5.23	3.4
3	Basal+H_3PO_4	32.3	198.9	0.30	0.15	0.45	1.56**	5.39	3.5

* In Experiments I, II, III, IV and V six pigs were individually fed *ad libitum* per treatment, and in Experiment VI twelve pigs were group fed per treatment.
** Significant at the 1% level.
* Significant at the 5% level.

tory. All chemical analyses of the bones were made of the head section of the right femur.

The practical corn-soy rations fed in Experiment VI are shown in table 5. Three lots of 12 weanling pigs each were fed, respectively, the corn-soy basal, the basal plus 0.15% phosphorus from dicalcium phosphate, and the basal plus 0.15% phosphorus from 75% concentrated phosphoric acid. Each lot was group fed *ad libitum* on concrete floored pens with outside exposure. Each animal was weighed weekly for the first 7 weeks and bi-weekly

thereafter. At the end of the feeding period each animal in lots 1 and 2 was bled for serum phosphorus analyses as previously described.

Bone breaking strength was determined at the Purdue University Materials and Structural Testing Laboratory in the following manner: The bones, which had been frozen since sacrifice, were completely thawed at room temperature. The center, in length of the bone, was determined and points 1.5 in. from the center were marked on each side of the center line. A hydraulic press (Rheile) was loaded at the center of this 3-inch span. The supports consisted of two transverse cylindrical steel rods seated in half-circle grooves, and held parallel at the fixed distance (3 in.) from each other by a solid block of wood. The bone was placed on the supports posterior side down. The diameter of the transverse rods and the rounded bearing surface of the loading bar was approximately 1 in. After the break, the wall thicknesses were taken at the thinnest and thickest edges of the break. These determinations were made on the femur bones from Experiment I only.

The data were treated statistically by the method of analysis of variance as outlined by Snedecor (1950).

Results

Growth Rate and Feed Efficiency

The growth and feed data are summarized in table 6.

Experiment I. In Experiment I there were no significant differences in growth rate or feed efficiency when dicalcium phosphate and "soft phosphate" were compared at the same dietary phosphorus level or at the three different levels. The pigs on the 0.3% level did just as well as those on either the 0.45 or 0.60% levels, of either supplement, according to analysis of the data for the entire feeding period. However, for the critical first 28 days the gains were 1.33, 1.39, 1.68, 1.52, 1.62 and 1.43 lb., respectively, for lots 1 through 6. These differences were not statistically significant because of animal variation but they indicate clearly that a level of 0.30% phosphorus was inadequate for maximum growth and also that the 0.60% level of "soft phosphate" depressed growth. Feed efficiency followed the same pattern as the growth rate.

Beginning about the seventh week of the experiment several pigs on each treatment were observed to have skeletal abnormalities. There was a general incidence of bowing out of the hind legs at the ham level, flabbiness of the hams, slightly sickle hocked and weak front and rear pasterns. These conditions were noted in the 0.30% "soft phosphate" lot one week earlier than in the others and became most severe in the 0.60% "soft phosphate" group. The following packing-house observations were made: (1) a few petchia on the kidney were observed in approximately 70% of the hogs, (2) all of the 36 had enlarged kidneys, (3) the lean meat of the hams was very pale,

and (4) the meat was spongy and the fat was flabby and watery, lacking firmness.

Experiment II. When the several supplements were compared at the same total phosphorus level, 0.30% in Experiment II, no significant differences were noted between treatments in average daily gain or feed efficiency in the final summary. But there were differences at 28 days as shown by the average daily gains of 1.49, 1.29, 1.36, 1.57, 1.57, and 1.40, respectively, for lots 1 through 6 at that time. From visual observations and daily gains during this early period it was obvious that the pigs fed the steamed bone meal, defluorinated phosphate, and "soft phosphate" (lots 2, 3 and 6) gained at a slower rate and developed more deficiency symptoms than those fed the other supplements. The lots receiving either dicalcium phosphate, monocalcium phosphate or Curacao Island phosphate did equally well in growth rate and feed conversion throughout the trial. On the 12th day one animal in lot 5 (Curacao) and two in lot 6 (soft phosphate) had a weakness in the hind legs characterized by a bowing out of the hams, sickle-hocked condition and weak pasterns. One week later a slight sickle-hocked condition was observed in one pig in lot 3 and two animals in lot 6. Thereafter more leg weaknesses appeared in all lots but the most severe cases were in lots 6 and 5, respectively. A general flabbiness of the hams appeared in all lots.

Experiment III. Data from Experiments I and II indicated that the basal ration was too high in phosphorus (0.18%) to permit a critical evaluation of the supplements when added to provide a minimal adequate phosphorus level (0.30%). Therefore, beef blood fibrin replaced the casein in the rations fed in Experiments III, IV, and V thus lowering the phosphorus in the basal to 0.035%. With this ration the animal response was much more rapid and more marked than previously.

In experiment III, feeding "soft phosphate" reduced growth rate ($P<0.05$), in comparison to the dicalcium phosphate-fed controls, and increased feed per pound of gain 0.21 lb. Commercial monocalcium phosphate was equal to dicalcium phosphate during the critical first 6 weeks but fell behind slightly the last 2 weeks. Deficiency symptoms began to appear in all lots during the fourth week. As the experiment progressed the symptoms became more severe in the "soft phosphate" group. Even though this group continued to gain in weight, skeletal growth appeared to be almost at a standstill near the end of the 56-day feeding period.

Experiment IV. Pig response was more marked in Experiment IV than before perhaps because younger and lighter pigs were used. Pigs in the "soft phosphate" group gained slower ($P<0.01$), ate less feed ($P<0.01$) and required more feed ($P<0.01$) per pound of gain than the dicalcium phosphate controls. Except for one pig in the Curacao group, this group did nearly as poorly as the "soft phosphate" group except for feed efficiency which was good. The addition of calcium fluoride to supply

the same fluorine level as was present in the "soft phosphate" ration (373 p.p.m.) did not affect pig performance in any way (lot 6). Definite leg weaknesses began to appear in the Curacao and "soft phosphate" groups during the second week of the experiment. These conditions became equally more severe in both groups and after 19 days on treatment one pig in each group could not stand. Several other pigs in each group were affected though less severely. They were sickle-hocked, hump-backed, pinched in the loin and had crooked front legs and weak pasterns. When the pigs were forced to walk they did so in pain and with a very strained gait. The pig which went down in the Curacao group was back on her feet in 7 days. However, the pig in the "soft phosphate" group which went down at the same time did not recover and was sacrificed after being down for 21 days. Two other pigs went down in the "soft phosphate" group and both died. The bones of all dead pigs were so soft that they could be severed with a knife.

The animals fed steamed bone meal were intermediate in general appearance between the Curacao group and either the dicalcium phosphate, monocalcium phosphate or the dicalcium phosphate plus sodium fluoride groups. Two pigs, especially, had severe leg and pastern weaknesses yet continued to eat and grow at a good rate and walked with much difficulty. Skeletal weaknesses appeared less severe in the latter groups.

On the 56th day all animals except two from each treatment (the most severe and an intermediate) were slaughtered for femur ash and fluorine analyses. These analyses showed a statistical significant lowering of the femur ash of the pigs fed "soft phosphate" and also a high fluorine content (see table 8). Over three times as much fluorine was present in the bones from the pigs fed "soft phosphate" as similar pigs fed the same level of fluorine as calcium fluoride (lot 6).

Several treatment changes were made during the next 98 days on the two pigs remaining from each treatment. First, the phosphorus content of the rations was raised to 0.45% using the original phosphorus sources. This resulted in a raised serum phosphorus and increased strength in the legs, but the crookedness of the legs did not change. Daily exercise and a two-hour exposure to sunshine did not effect an improvement; neither did substitutions of yellow corn for the cerelose and starch, and dried skimmilk for the blood fibrin for a 42-day period improve the crooked legged condition. Analyses of the femurs collected on the 98th day of the recovery phase showed no treatment differences. The ash and phosphorus values were approximately 26 and 4.5%, respectively, of the fresh sample weight.

Experiments V and VI. Both Experiments V and VI show that pigs receiving phosphoric acid did as well as those receiving dicalcium phosphate at the same level on either semi-purified or a corn-soy diet. On the semi-purified diet (Experiment V) the phosphoric acid-fed group did slightly better throughout in both growth rate and general appearance. It appeared

that the diets containing phosphoric acid were more palatable than those containing dicalcium phosphate as evidenced by a very marked increase in feed consumption the first 2 weeks in both experiments. Pigs on the corn-soy diet, without phosphorus supplementation, gained at a slower rate ($P<0.01$), ate less feed and required more feed per pound of gain than those with phosphorus supplementation (lots 2 and 3), showing that such a corn-soy diet is inadequate in available phosphorus for good growth and feed efficiency of fattening swine.

Serum Phosphorus

Considerable variation is shown in serum phosphorus values in Experiments I and II, table 7. However, the data from Experiment I indicate that the more phosphorus in the diet the more will be absorbed into the blood. For the first 6 weeks there was an increase in serum phosphorus with each increase in dietary phosphorus from 0.30 to 0.60%. In Experiments III and IV, when most of the dietary phosphorus was supplied by the supplement being tested, "soft phosphate" or Curacao did not maintain the serum phosphorus as well as the other supplements. The differences between these and the dicalcium phosphate controls were highly significant ($P<0.01$). Also, in Experiment V dicalcium phosphate did not maintain serum phosphorus levels as high as phosphoric acid fed at the same phosphorus level. These differences approached significance. Pigs fed a corn-soy ration containing 0.30% phosphorus for 111 days had a much lower serum phosphorus ($P<0.01$) than those fed the same ration supplemented with 0.15% phosphorus as dicalcium phosphate.

Bone Data

The data in table 8 show considerable accumulation of fluorine in the bones of the animals fed "soft phosphate". The flourine accumulation increased as the amount of "soft phosphate" in the feed was increased. The younger and lighter pigs used in Experiment IV accumulated more fluorine in a shorter period of time than the larger pigs used in Experiment I. A comparison of lots 5 and 6 in Experiment IV shows that over three times more fluorine was deposited in the bones of pigs in the "soft phosphate" group than pigs receiving the same amount of fluorine as U.S.P. grade calcium fluoride added to the dicalcium phosphate basal. This indicates that the fluorine in "soft phosphate" is more easily absorbed and deposited than the fluorine in calcium fluoride. The pigs which received 69 p.p.m. fluorine from Curacao deposited more fluorine in the femurs than did the pigs which received 373 p.p.m. fluorine from calcium fluoride (0.47 and 0.36% fluorine in the ash, respectively).

The ash analyses showed no treatment differences when the casein basal ration (0.18% phosphorus) was used (Experiments I and II), but

a marked lowering by the "soft phosphate" when the low phosphorus (0.035%) fibrin basal was used. The difference between the "soft phosphate" group and any other in Experiment IV was highly significant ($P<0.01$).

TABLE 7. BLOOD SERUM INORGANIC PHOSPHORUS AS AFFECTED BY TREATMENT

(MG. P PER 100 ML. SERUM)

Treatment	Lot	Days on treatment				
		0	14	28	42	56
	Experiment I[a]					
Dicalcium phosphate (0.30% P)	1	8.2	6.7	7.6	4.7	4.7
Soft phosphate (0.30% P)	2	8.0	7.7	8.2	5.0	5.6
Dicalcium phosphate (0.45% P)	3	8.4	8.3	9.7	5.7	5.9
Soft phosphate (0.45% P)	4	6.1	7.8	9.1	6.0	6.7
Dicalcium phosphate (0.60% P)	5	8.1	10.0	10.0	6.4	5.6
Soft phosphate (0.60% P)	6	8.0	9.9	10.9	6.8	6.3
	Experiment II					
Dicalcium phosphate	1	6.6	6.8	6.5	7.5	8.5
Steamed bone meal	2	7.1	6.6	5.7	6.6	7.5
Defluorinated phosphate	3	6.3	6.2	6.1	5.9	6.9
Monocalcium phosphate	4	5.8	7.4	8.0	7.7	7.3
Curacao Island phosphate	5	5.6	7.2	7.1	6.6	7.0
Soft phosphate	6	4.9	7.0	7.9	6.6	6.9
	Experiment III[b]					
Dicalcium phosphate	1	6.0	7.1	7.1	7.4	7.6
Monocalcium phosphate	2	6.8	6.9	7.2	7.4	7.7
Soft phosphate with colloidal clay**	3	5.8	5.1	5.3	5.5	6.5
	Experiment IV[b]					
Dicalcium phosphate	1	5.7	6.9	6.8	8.0	7.7
Monocalcium phosphate	2	5.3	7.3	7.6	8.4	8.2
Steamed bone meal	3	5.7	6.2	7.5	7.4	7.3
Curacao Island phosphate**	4	5.4	5.3	5.8	6.8	6.7
Soft phosphate with colloidal clay**	5	6.1	5.6	6.1	5.4	5.3
Dicalcium phosphate+CaF_2	6	6.7	7.4	8.2	8.8	9.5
	Experiment V					
Dicalcium phosphate	1	8.8	4.8	5.8	6.5	6.8
H_3PO_4	2	7.1	6.5	7.7	7.5	8.3
	Experiment VI[c]					
Basal	1	...	...	...	...	6.0**
Dicalcium phosphate	2	...	...	...	...	8.1
H_3PO_4	3	...	...	...	...	...

[a] After 90 days on treatment, all lots were approximately equal (9.5, 9.4, 9.3, 9.3, 8.8 and 9.7 mg., respectively).

[b] The first blood samples for Experiments III and IV were drawn on the second day of the experiment.

[c] Lots 1 and 2 were bled on 111th day of the experiment, 4 days after lot 3 reached market weight.

** Averages were lower than the dicalcium phosphate controls ($P<0.01$).

Breaking Strength of Femurs

The pounds of pressure required to break the femur bone was measured on one bone from each animal in Experiment I. The averages for lots 1, 2, 3, 4, 5, and 6, respectively, were 829, 703, 1015, 816, 865, and 810 lb. The dicalcium phosphate lots, 1, 3, and 5 required more pressure than the corresponding "soft phosphate" lots, 2, 4, and 6. The difference between lots 3 and 4 was significant ($P<0.05$).

TABLE 8. CHEMICAL ANALYSES OF THE HEAD SECTION OF FEMURS (AVERAGE BY TREATMENT)

Lot	Treatment	Ash [a]	Phosphorus [a]	Flourine [b]
		%	%	%
	Experiment I—90 days			
1	Di-Cal (0.30% P)	36.15	6.47	0.029
2	Soft phos. (0.30% P)	35.40	6.33	0.507
3	Di-Cal (0.45% P)	38.30	6.88	0.028
4	Soft phos. (0.45% P)	36.65	6.55	0.778
5	Di-Cal (0.60% P)	37.10	6.73	0.039
6	Soft phos. (0.60% P)	37.55	6.91	1.17
	Experiment II—63 days			
1	Di-Cal	35.75	6.48	0.049
2	Bone meal	36.5	6.66	
3	Defluor. phos.	36.1	6.63	
4	Mono-Cal	36.2	6.60	
5	Curacao	35.9	6.52	0.134
6	Soft phos.	34.2	6.10	0.502
	Experiment IV—56 days			
1	Di-Cal	49.97		.04
2	Mono-Cal	50.61		.05
3	Bone meal	46.30		.10
4	Curacao	46.67		.47
5	Soft phos.	36.18**		1.23
6	Di-Cal+CaF_2	51.47		.36

[a] Ash and phosphorus for Experiments I and II are expressed as percent of the fresh bone and for Experiment IV as percent of the dry fat-free bone.
[b] Flourine is expressed as percent of the ash of the pooled samples.
** Significant at 1% level.

Teeth.

Observations made of the teeth of the lower mandible of all animals in Experiment I at sacrifice (after 90 days on the respective treatments) are summarized in table 9. "Soft phosphate" resulted in more pitting and decay than dicalcium phosphate at all three levels compared. All animals in the 0.60% "soft phosphate" lot had pitting and excessive decay as compared to no decay and only two cases of pitting and no excessive decay in the 0.60% dicalcium phosphate group.

Discussion

The results of this study emphasize the fact that in order to critically evaluate phosphorus supplements for swine the basal rations to which the supplements are to be added should be as low in phosphorus as possible and also that each supplement should be added in amounts to provide a minimal adequate total phosphorus level. In other words, the more inadequate the basal ration in phosphorus the greater the possibility that the animal will respond to any given dietary addition of phosphorus in an available form and once the animals needs are met any further additions cannot be expected to produce a positive response. A comparison of Experiments I and II with III, IV, and V shows this very clearly. Thus,

TABLE 9. OBSERVATIONS OF THE TEETH OF THE LOWER MANDIBLE (EXPERIMENT I, SIX ANIMALS PER TREATMENT)

Treatment	Number of animals in each treatment exhibiting		
	Mottling	Pitting	Excessive decay
0.30% dicalcium phosphate	6	2	0
0.30% "soft phosphate"	4	3	3
0.45% dicalcium phosphate	3	3	0
0.45% "soft phosphate"	5	2	3
0.60% dicalcium phosphate	4	2	0
0.60% "soft phosphate"	1	6	6

the poor results with "soft phosphate" agree with those of Chapman *et al.* (1956) and are in disagreement with those of Gobble *et al.* (1956). Essentially, these results agree with the many detailed studies which have been reported with poultry, and especially with the results obtained by Gillis (1954). Gillis tested the different samples of the many products separately while our comparisons were made of the composites of the different samples.

A level of 0.30% phosphorus was inadequate for normal skeletal growth as evidenced by the fact that at least one-half of all animals on Experiments I through V developed skeletal weaknesses during the experiment. In a majority of cases the abnormalities reached their maximum degree during the first 6 weeks of the feeding period and gradually improved thereafter. The degree of improvement was reflected in an increase in the serum inorganic phosphorus values. Generally, in a given treatment, the animals which ate the most feed and gained at the fastest rate developed the most severe skeletal abnormalities.

Figures 1 and 2 show the most common visual symptoms noted.

A possible criticism of these data may be that semi-purified diets low or void in natural phosphatase enzymes were used. Several workers,

Figure 1. A typical phosphorus-deficient pig is shown on the left. The pig on the right received the same ration adequate in available phosphorus.

the most recent being Hill and Taylor (1954), have shown that natural feedstuffs vary considerably in phytase content and that an animals ability to utilize phytin phosphorus may vary directly with the amount of feed phytase present. However, no such enzyme relationship to phosphorus utilization has been shown with the inorganic phosphorus sources used in these studies.

Even though the results show that the phosphorus in phosphoric acid is highly available to swine, more research is needed before its use in practical rations is recommended. More should be known about ways of mixing it into different formulas, its effect on different nutrients in the formulas as affected by time and method of storage, its effect on different storage containers, and the possible hazards to personnel and equipment. In these studies, all mixed feed was stored in cardboard drums and was fed within 2 weeks after mixing. Crude estimates of the pH showed that the feed was still slightly acid after 2 weeks of storage. Of course, this would vary with different formulas depending mainly upon the amount of the acid added and the amount of other minerals, particularly calcium, present.

Figure 2. Slow growth and collapse of the rear quarters characterized this pig which developed severe phosphorus deficiency.

The samples of soft phosphate used in these studies contained an average of 1.51% fluorine which supplied approximately 655 p.p.m. fluorine to lot 6 of Experiment I, 187 p.p.m. fluorine to lot 6 in Experiment II and 373 p.p.m. fluorine to the "soft phosphate" lots in Experiments III and IV. With the exception of the excessive pitting and decay of the teeth in the high "soft phosphate" group of Experiment I, the poor results obtained with "soft phosphate" in this study appear to be due to the poor availability of the phosphorus rather than to its fluorine content. This seems true in spite of the fact that both the 373 and 655 p.p.m. levels fed were considerably above the safe levels of 100 to 200 p.p.m. recommended by the National Research Council (1955). The leg weaknesses, reduction in growth rate and in serum phosphorus levels appeared too early in the trials and appeared to be too closely associated to have resulted from an excess of fluorine. Since over three times as much flourine was deposited in the femurs from feeding 373 p.p.m. of fluorine as "soft phosphate" than from feeding the same level of fluorine as U.S.P. grade calcium fluoride, it seems that a considerable amount of the fluorine in "soft phosphate" is in a form other than calcium fluoride and more probably as sodium fluoride. The data indicate that the fluorine in Curacao was also more readily absorbed than the fluorine in calcium fluoride.

Even though a practical diet of corn and soybean oil meal supplemented with calcium, antibiotic, vitamin B_{12} and B-vitamin supplements, and containing approximately 0.30% phosphorus was deficient in this element as measured by growth rate, feed efficiency, and serum phosphorus analyses, no skeletal abnormalities appeared. On the purified diets containing the same level of phosphorus from any source, leg and other skeletal abnormalities developed. The possibility that unidentified factors associated with bone metabolism were present in the practical diets and lacking in the semi-purified diets cannot be discounted. Aside from the diets, two other differences should be noted. Firstly, the pigs fed the purified diets ate more feed and gained at a much faster rate for their size than those fed the natural diets. This was especially true during the first 4 weeks of every trial. Secondly, all semi-purified diets were fed indoors and the practical corn diet was fed outside on concrete floors.

The pale color of the lean tissue of the hams and the "watery", "flabby" character of the fat tissue of the hams and bacon in Experiments II and III, in all probability, were the result of the high sugar content of the rations used. Similar observations have been made in other studies at this station in which high cerelose diets were used. Also, enlarged kidneys and petechia of the kidneys have been observed in previous experiments. Increased water intake and diuresis were observed in some of the animals. Since neither water intake or excretion were measured the cause is not clear but most likely was due to one or a combination of the following: the phosphorus level or source, the fluorine intake, or the amount of cerelose

consumed. Differences in water intake were less noticeable in Experiments III, IV, V and VI, than in I and II in which high cerelose diets were fed. Riddell *et al.* (1934) reported that cows on a low phosphorus ration consumed more water than cows receiving the same ration supplemented with phosphorus. Aubel *et al.* (1936) observed that feeding low phosphorus rations to pigs increased thirst and urinary excretion, and enlarged kidneys which showed signs of chronic nephritis when examined histologically. Rations containing large amounts of fluorine caused an increased water consumption and a diuresis in pigs (Kick *et al.* 1935).

Summary

Six dry-lot feeding experiments were conducted to determine the availability of the phosphorus in various phosphate materials for growing swine. Semi-purified rations were used in five experiments and a practical corn-soybean oil meal ration was used in one experiment. In the semi-purified diet studies six pigs were fed individually *ad libitum* per treatment on indoor concrete floors. The pratical diet was self-fed to a group of 12 pigs per treatment on concrete floors with outside exposure.

The criteria of evaluation were: growth rate, feed consumption, feed per unit gain, bi-weekly inorganic phosphorus analyses, femur ash, phosphorus and fluorine analyses and visual observations.

The supplements ranked in the following order: dicalcium phosphate, monocalcium phosphate, and phosphoric acid about equal followed by steamed bone meal and defluorinated phosphate, then Curacao Island phosphate and finally soft phosphate with colloidal clay the poorest.

In comparisons of "soft phosphate" with dicalcium phosphate at the same phosphorus level the former resulted in a highly significant reduction in gains, feed intake, serum phosphorus, and ash content of the femurs. The fluorine content of the femurs was increased from 16 to 30 times by feeding "soft phosphate". Adding "soft phosphate" to a basal ration containing 0.18% phosphorus to supply a total of 0.60% resulted in excessive pitting and decay of the molars as compared to the same level of phosphorus from dicalcium phosphate. Three times as much fluorine was deposited in the femurs from feeding "soft phosphate" as from feeding the same amount of fluorine as calcium fluoride added to the dicalcium phosphate basal.

In one experiment Curacao was equal to dicalcium phosphate but in a later more critical test using the extremely low phosphorus basal (0.035% P) the Curacao pigs ate less, gained slower, had lower serum phosphate values and developed more severe skeletal abnormalities than the dicalcium phosphate controls.

Pigs fed steamed bone meal developed more leg weaknesses than the dicalcium phosphate controls, yet gains were similar. Defluorinated phosphate was about equal to steamed bone meal.

Commercial monocalcium phosphate was equal to dicalcium phosphate in two purified-diet tests.

Analytical grade phosphoric acid was equal to U.S.P. grade dicalcium phosphate in all criteria measured in one purified-diet study and in one study using a corn-soybean oil meal basal ration.

Adding 0.15% phosphorus from either dicalcium phosphate or phosphoric acid to a practical corn-soybean oil meal ration (0.30% phosphorus) resulted in a highly significant increase in rate of gain and serum phosphorus values and also a marked increase in feed efficiency.

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THE PHOSPHORUS REQUIREMENT OF CALVES

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ALTHOUGH a considerable amount of research has been conducted to determine the phosphorus requirement of animals, the calf under 1 year of age has received little attention. Henderson and Weakley (1930) reported that dairy calves fed 0.13% phosphorus with an adequate level of calcium grew as well as calves fed 0.22%; however, low serum inorganic phosphorus levels and bone ash were exhibited at lower phosphorus level. Jones *et al.* (1934), working with 8-month old dairy heifers reported an increase in growth after increasing the dietary phosphorus level from 0.26% to approximately 0.36%. Huffman *et al.* (1933) stated that 0.20% of dietary phosphorus was insufficient for satisfactory growth in dairy heifers from 3 to 18 months of age, however, 0.41% appeared sufficient. From a similar study involving two groups of 6-month old calves, Archibald and Bennett (1935) concluded that about 0.18% phosphorus was as effective as 0.31% in promoting growth. Beeson *et al.* (1940) found that 0.18% dietary phosphorus was adequate for satisfactory weight gains and blood phosphorus levels in yearling beef steers; however, Burroughs *et al.* (1956) observed an increase in feed lot performance when the phosphorus in the ration was raised from 0.18% to 0.25%. Long *et al.* (1956) reported linear increases in feed intake, weight gains and plasma inorganic phosphorus levels over the range of 0.07, 0.11 and 0.15% dietary phosphorus in yearling beef steers. Slight increases in gain were observed when the phosphorus level was further increased to 0.19%. The National Research Council (1950) recommended a phosphorus level of 0.40% of the air-dried ration for dairy calves weighing 200 lb.

The lack of agreement among the previous estimates of the phosphorus requirements of cattle and the paucity of information pertaining to the young growing calf is evident. This work was designed to determine more precisely the phosphorus requirement of the calf between 3 and 6 months of age.

Experimental Procedures

Forty Holstein male calves were used in two experiments. Experimental procedures for both experiments, except for phosphorus levels fed, were similar and will be presented together. Calves were purchased at 2 to 3 days of age and fed a milk, calf starter, alfalfa hay ration. At 12 weeks of

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TABLE 1. COMPOSITION OF BASAL RATION [a]

Component	% of air-dried ration
Degerminated corn meal	40.0
Beet pulp and dried molasses	15.0
Cerelose (Glucose·H_2O)	15.0
Corn oil	5.0
Corn starch	13.458
Blood meal	9.0
Urea	0.5
NaCl	1.00
$CaCO_3$	1.00
Trace minerals [b]	0.042

[a] Vitamin A and D oil was added to supply 3375 I.U. of vitamin A activity and 650 I.U. of vitamin D per pound of feed.

[b] The trace mineral mixture supplied 20 mg. iron, 10 mg. copper, 10 mg. manganese and 1 mg. cobalt per pound of feed.

age they were gradually changed to a low-phosphorus semi-purified basal ration, the composition of which is shown in table 1.

The basal ration, analyzed according to the method of Koenig and Johnson (1942), contained 0.09% phosphorus for Experiment I and 0.10% phosphorus for Experiment II. Each experiment consisted of 4 treatments (phosphorus levels) with 5 calves per treatment. Desired phosphorus levels were obtained by addition of dicalcium phosphate to the basal ration. The calcium and phosphorus levels and the Ca:P ratios of the rations are presented in table 2. The calves were individually fed *ad libitum* throughout the 42-day test period.

Daily feed intakes were recorded and weight gains taken at bi-weekly intervals. Blood was sampled by jugular puncture initially and at 14-day intervals thereafter. Serum inorganic phosphorus levels were determined by the molybdivanadate method of Simonsen *et al.* (1942) and serum alkaline phosphatase activity was calculated by the Bodansky (1933) method adapted to the use of the molybdivanadate method for measuring the phosphorus released.

Autoradiographs were employed to measure bone growth using the

TABLE 2. PHOSPHORUS LEVELS, CALCIUM LEVELS AND Ca:P RATIOS OF RATIONS FED

Experiment I (0.09% P in basal ration)				
Total phosphorus, %	0.09	0.12	0.18	0.30
Total calcium, %	0.48	0.52	0.60	0.77
Ca:P ratios	5.33	4.33	3.33	2.57
Experiment II (0.10% P in basal ration)				
Total phosphorus, %	0.14	0.22	0.30	0.38
Total calcium, %	0.55	0.55	0.55	0.65
Ca:P ratios	3.93	2.50	1.83	1.71

distal ends of the femurs from all calves and in addition the costal ends of the eighth rib of calves from Experiment II. Two millicuries of Ca^{45} were injected intravenously at the beginning of the experimental period. After 42 days, the calves were killed and the bones removed. Longitudinal sections from the center of the bones were cleared of dust and fat with alcohol and ether and exposed for a period of 4 to 6 days on X-ray film. After developing the film, femur growth area was measured with a planimeter and the growth in length of the ribs was taken with vernier calipers.

Ash content of a 1-inch sample of the dry, fat-free ninth rib (costal end) was determined (A.O.A.C., 1945). Other criteria which appeared

TABLE 3. AVERAGE RESPONSES OF CALVES FED DIFFERENT PHOSPHORUS LEVELS—EXPERIMENT I

Criteria	Phosphorus level, %			
	0.09	0.12	0.18	0.30
Initial weight, lb.	193.2	191.0	194.2	196.2
Total gain, lb.	34.8	38.0	57.4	64.2
Total feed intake, lb.	202.8	202.7	241.5	252.2
Feed/gain[a]	6.37	5.65	4.28	3.97
Serum inorganic phosphorus				
Change, mg. %	−3.22	−2.83	−1.20	2.11
Terminal, mg. %	4.32	4.35	5.86	9.17
Serum phosphatase activity				
Change, Bodansky U.	0.21	1.75	0.60	−1.27
Terminal, Bodansky U.	11.25	10.87	11.90	8.93
Femur growth, sq. in.	0.831	0.823	1.015	1.083
Ash in rib, %	50.36	48.30	49.45	53.65

[a] Calculated as an average of individuals.

insensitive to changes in dietary phosphorus were increase in height at the withers, weight of the entire ninth rib, percentage of phosphorus in the rib and in the rib ash.

The data collected for each criterion were analyzed statistically by analysis of variance and treatment means were subjected to the multiple range test of Duncan (1955).

Results

Growth and feed intake on the semi-purified basal ration supplemented with an adequate amount of phosphorus compared favorably with calves fed natural rations, as judged by the Ragsdale standard (Peterson, 1950). This indicates that the basal ration was nutritionally adequate except for phosphorus. Average responses of calves fed the different phosphorus levels are presented in table 3 for Experiment I and in table 4 for Experiment II. A summary of the mean squares from the analyses of variance of data in both experiments is shown in table 5.

Experiment I

Growth Study. Feed intake means (table 3) indicated that consumption was directly related to levels of dietary phosphorus. Biweekly feed intake decreased from an initial consumption of approximately 80 lb. to less than 70 lb. per animal on the rations containing 0.09 or 0.12% phosphorus, whereas consumption increased on the two higher levels so that calves fed the 0.30% phosphorus level ate over 90 lb. of feed each during the last two weeks of the experiment. Weight gains increased with increases in dietary phosphorus over all treatments (table 3). Statistical analysis

TABLE 4. AVERAGE RESPONSES OF CALVES FED DIFFERENT PHOSPHORUS LEVELS—EXPERIMENT II

Criteria	Phosphorus level, %			
	0.14	0.22	0.30	0.38
Initial weight, lb.	212.0	213.6	210.0	214.4
Total gain, lb.	37.0	51.0	51.8	49.0
Total feed intake, lb.	218.0	243.2	245.0	240.2
Feed/gain[a]	6.60	4.94	4.89	4.93
Serum inorganic phosphorus				
Change, mg. %	−5.35	−3.53	−2.35	−1.80
Terminal, mg. %	4.90	6.70	8.35	8.06
Serum phosphatase activity				
Change, Bodansky U.	9.94	7.25	7.15	2.47
Terminal, Bodansky U.	18.05	14.45	14.93	9.62
Femur growth, sq. in.	0.78	1.00	0.93	0.96
Rib growth, cm.	2.16	2.48	2.35	2.42
Ash in rib, %	45.63	48.86	51.44	50.05

[a] Calculated as an average of individuals.

revealed a significant difference in gains attributable to treatment ($P<0.01$). Figure 1 represents cumulative weight gains the 42-day experimental period. Calves fed the three lower phosphorus levels gained at a slower rate; however, growth on the 0.18% level approached that on the highest level. This indicated that 0.18% phosphorus was slightly less than the minimum requirement of the calves. Feed conversion values (table 3) showed that efficiency of feed utilization increased as the level of phosphorus increased to the point of an adequate amount in the ration. From this experiment it was not possible to conclude that the 0.30% level was entirely adequate; however, the 0.09, 0.12 and 0.18% phosphorus levels were all shown to be insufficient for optimum feed conversion.

Blood and Bone Studies. Serum inorganic phosphorus levels changed rapidly as a reflection of changes in dietary phosphorus. Calves on all treatments averaged approximately 7 mg.% at the beginning of the experimental period; however, when the experiment was terminated the calves on the 0.30% level of dietary phosphorus averaged about 9 mg.% while

TABLE 5. SUMMARY OF MEAN SQUARES FROM THE ANALYSES OF VARIANCE OF GROWTH, FEED, BLOOD AND BONE DATA

Source of variation	Degrees of freedom	Weight gain	Feed intake	Feed / gain	Change in serum phosphorus[a]	Change in serum phosphatase[a]	Femur growth	Rib growth	% Ash in rib
				Experiment I					
Replication	4	116.8	1492	1.23	0.68	19.14**	0.008	.....	5.11
Treatments	3	1057.7**	3338	6.42*	29.45**	7.66	0.086*	.....	26.42**
Expt. error	12	135.9	1192	1.45	0.73	2.47	0.015	.....	3.24
				Experiment II					
Replication	4	129.7	956	1.82	1.29	6.29	0.012	0.073**	5.37
Treatments	3	238.1	792	3.51	12.34*	48.09**	0.044**	0.097**	30.48*
Expt. error	12	125.1	364	2.22	2.62	6.23	0.004	0.010	6.14

[a] Analysis of variance was conducted on coded values. Coded values were derived by adding 10 to original observations.
** Significant at 1% level of probability.
* Significant at 5% level of probability.

the 0.18% calves averaged about 6 mg.% serum phosphorus and calves fed the two lower levels exhibited terminal values approximating 4 mg.% (table 3). Serum alkaline phosphatase activity at biweekly intervals showed some interesting trends. During the first 2-week period there was an increase in activity in the calves fed the 0.09, 0.12 and 0.18% levels of phosphorus but a decrease in activity in calves that received 0.30% phosphorus. In all cases increased phosphatase activity accompanied a decreased serum inorganic phosphorus level and *vice-versa*. After the

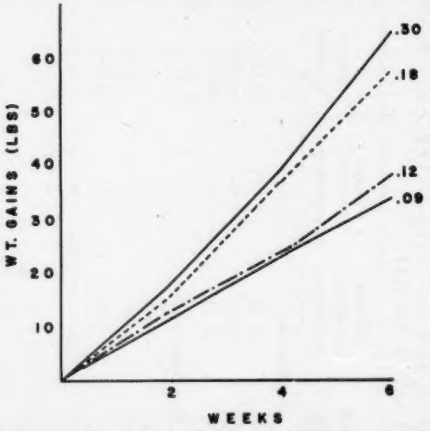

Figure 1. Cumulative weight gains of calves fed different levels of phosphorus. The values on the right side of the graph represent percent phosphorus in the air-dried ration. Experiment I.

initial 2-week period, phosphatase activity tended to approach initial levels. These trends indicate that phosphatase activity responds rapidly to changes in the blood supply of phosphorus, but after the initial change again seeks its "normal" level.

The methods used in preparing the bone autoradiographs gave a well defined area of growth which could be measured with a high degree of precision (figure 2). The analysis of variance of femur growth data revealed a significant difference among treatment means ($P<0.05$), the greatest difference existing between the two low levels (0.09 and 0.12) compared to the 0.18% phosphorus level (table 3). Lack of statistical significance in the difference in growth between calves fed the 0.18 and 0.30% levels suggests that 0.18% phosphorus closely approached the minimum phosphorus requirement for calves of this age and weight.

Bone ash has been widely used as a measure of phosphorus sufficiency, especially in the case of chicks (Wilgus, 1931 and Gillis *et al.*, 1954).

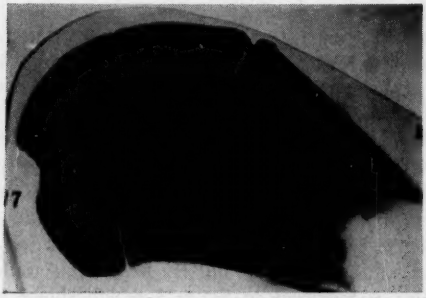

Figure 2. A representative femur autoradiograph. The calf was from Experiment I and received 0.30% phosphorus.

Although the difference in percentage of ash in the ribs of calves in this experiment was significant ($P<0.01$) most of the treatment variance arose from the 0.30% level compared to the other three which showed no significant difference (table 3).

Depraved appetite resulting from phosphorus deficiency has been reported by several workers (Theiler, 1927; Becker *et al.*, 1933; Beeson *et al.*, 1944). After 2 to 3 weeks, calves on the 0.09, 0.12 and 0.18% phosphorus levels began chewing the wood stalls. Only 1 calf on the 0.30% level was observed in the act of wood chewing. Toward the end of the experiment calves fed the 0.09 and 0.12% phosphorus levels assumed an unthrifty appearance, were listless and had rough hair coats.

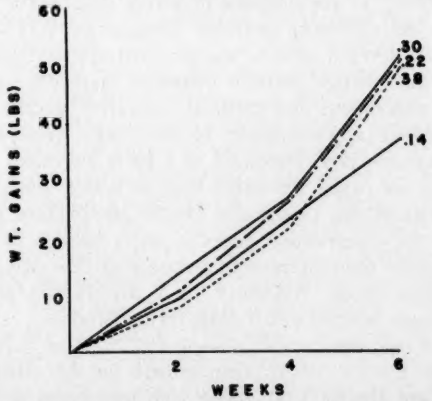

Figure 3. Cumulative weight gains of calves fed different levels of phosphorus. The percentages of phosphorus in the air-dried ration are shown on the right-hand side of the graph. Experiment II.

Experiment II

Growth Study. The average feed intake (table 4) of calves fed 0.22, 0.30 and 0.38% of phosphorus was very similar. The calves which received 0.14% of phosphorus consumed an average of about 25 lb. less feed than those fed the three higher levels over the 6-week experimental period. This difference was just short of significance at the 5% probability level. As with feed intake, body weight gains on the three high phosphorus levels were practically the same and represented a sizable increase over the calves fed the 0.14% level of phosphorus (table 4). Although the difference in total weight gains was slightly less than significant (table 5) the reason for this is apparent in figure 3 which shows the cumulative weight gains over the 42-day growth period. Here it is noted that a major portion of the total difference among treatments was due to responses during the last two weeks of the experimental period. The weight gain data indicate no advantage from supplying phosphorus in excess of 0.22% of the air-dried ration. Calves fed the 0.14% level of phosphorus required 6.6 lb. of feed per pound gain while those fed either 0.22, 0.30 or 0.38% phosphorus need approximately 4.9 lb. of feed for each pound of gain (table 4).

Blood and Bone Studies. Initial serum inorganic phosphorus values were considerably higher in this experiment than in Experiment I due to the fact that initial blood samples were drawn in Experiment II while the calves were still receiving the phosphorus-rich (0.70%) calf-starter. In the first experiment blood samples were drawn after the calves had been fed the unsupplemented basal ration for a period of 5 days. A significant difference ($P<0.05$) in the changes in serum phosphorus levels arising primarily from the difference between changes on 0.14% and 0.22% dietary phosphorus levels (table 4) was revealed in the statistical analysis. Serum alkaline phosphatase activity increased markedly during the first 4 weeks of the experiment and declined thereafter. Increases in activity for this period were approximately 11 Bodansky units for calves fed the 0.14% phosphorus level compared to 8 units for calves on the 0.22% level and 5 units for calves fed either 0.30 or 0.38% phosphorus. In the statistical analysis of the changes in serum phosphatase activity which took place over the experimental period a major portion of the treatment variation arose from the difference in response of this enzyme to the two highest phosphorus levels. Evidently there are factors affecting serum alkaline phosphatase activity which merit further study.

Femur growth on the 0.14% phosphorus level of 0.78 square inch was significantly less ($P<0.01$) than growth on the other three levels. The largest growth area of 1.00 square inch was found in calves fed the 0.22% level of phosphorus; however, this did not prove to be significantly different from the 0.93 square inch recorded for calves fed the 0.30% level or the 0.96 square inch area from those fed 0.38% of phosphorus.

No significant differences in rib growth were detected in calves that received phosphorus levels equal to or exceeding 0.22% of the ration (tables 4 and 5), whereas 0.14% phosphorus gave significantly less growth ($P<0.01$). Statistical treatment of rib-ash data revealed significance in the variation among treatment means ($P<0.05$). Multiple range testing (Duncan, 1955) showed that the variation could be attributed to the low ash levels in calves on the 0.14% phosphorus ration, the three higher levels showing no significant differences (table 4).

As in the previous experiment calves on the 0.14% phosphorus level developed rough hair coats and appeared unthrifty, whereas calves on the three higher phosphorus levels appeared vigorous and healthy.

Discussion

Considering both studies it is evident that the phosphorus requirement of calves weighing 200 to 275 lb. at an age of 12 to 18 weeks approximates 0.22% of the air-dried ration. In the first experiment, calves fed 0.30% phosphorus made greater gains in body weight, maintained their serum inorganic phosphorus at a higher level, had a higher percentage of ash in the rib bones and exhibited more bone growth than calves fed lower phosphorus levels. The difference in response to the 0.30 and 0.18% levels was not large in most instances, indicating that the phosphorus requirement might be between 0.18% and 0.30% of the ration. The second experiment showed that responses of the calves in terms of the above criteria as well as rib growth were not improved by additions of phosphorus above 0.22% of the ration.

A factor which could conceivably influence an estimate of the phosphorus requirement is the availability of this element from the sources used to supply the nutrient. The work of Reid and Franklin (1947) and Jordan *et al.* (1906) indicates that phytin is completely hydrolyzed by the ruminant releasing the phosphorus in the inorganic form, hence the presence of some phytin phosphorus in the basal ration was not considered a problem in the interpretation of results presented in this report. Dicalcium phosphate, used as the supplement in this study, has in many instances been shown to contain phosphorus in a form highly available to cattle (Burroughs *et al.*, Long *et al.*, 1956; Tillman *et al.*, 1956).

In practice the variation in animals and feedstuffs necessitates an increase in the phosphorus level of the ration, often referred to as a "safety factor". After due consideration the addition of a "safety factor" of 0.08% appears reasonable. This would increase the experimentally determined minimum requirement of 0.22% phosphorus to a level of 0.30% for calves 12 to 18 weeks of age and weighing from 200 to 275 lb.

As an item of added interest, the data accumulated in this study were subjected to further analysis as an aid in judging the relative precision of the several measurements used. In table 6 is given the average coef-

ficient of variation of several response criteria. Noteworthy is the large variation in changes in serum inorganic phosphorus as contrasted to low variation in the measurement of bone growth as here used and ash and phosphorus concentration of bones. Gains in weight were intermediate and at a level usually observed.

Summary

Forty Holstein male calves were used in two experiments to determine the phosphorus requirement. At 12 weeks of age the calves were changed from a grain-hay pre-experimental ration to a semi-purified basal ration containing 0.09–0.10% phosphorus. Dicalcium phosphate was added to the basal ration to supply total phosphorus levels on an air-dried basis of 0.09, 0.12, 0.18 and 0.30% in Experiment I. In Experiment II the dietary phosphorus levels were 0.14, 0.22, 0.30 and 0.38%. Twelve different response criteria were utilized and among them were feed intake and efficiency, body weight gains, serum inorganic phosphorus and alkaline phosphatase activity, bone ash, and bone growth as measured from femur and rib autoradiographs.

Autoradiographs proved to be a sensitive, precise and accurate technique for measuring the growth of the long bones. It appears that this method might be used to determine the adequacy of other nutrients which affect bone growth.

From these studies it is evident that the minimum phosphorus requirement of calves weighing from 200 to 275 lb. at 12–18 weeks of age approximates 0.22% of the air-dried ration. This means that feeding standards recommending as high as 0.40% phosphorus for calves of this size may be safely scaled downward. Adding a factor of safety to the minimum requirement, it is recommended that 0.30% phosphorus be included in the ration of calves of the size and weight employed in these studies.

TABLE 6. COEFFICIENTS OF VARIATION OF SOME RESPONSE CRITERIA USED IN THIS STUDY

	%
Total feed intake	14
Body weight gains	24
Feed/gain	26
Change in serum phosphorus	51
Change in serum phosphatase	37
Femur growth (autoradiographs)	10
Rib growth (autoradiographs)	8
Ash in rib	5
Phopshorus in rib ash	5

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DICALCIUM PHOSPHATE AND PHOSPHORIC ACID AS PHOSPHORUS SOURCES FOR BEEF CATTLE

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THE wintering rations of beef cattle usually contain a high proportion of mature and dried roughages. In general these roughages contain more calcium than phosphorus and in many cases an all roughage ration will supply sufficient calcium to meet the requirements of the animals. The commercially available mineral supplements used to supply phosphorus also contain as much or more calcium than phosphorus. It would be desirable to have an ingredient that would supply phosphorus without having to add other elements not needed in the ration. There are indications that phosphoric acid, supplying a unit of phosphorus at a lower cost than other supplements (Mehring, 1956), offers possibilities in this direction. Preliminary studies by Richardson *et al.* (1955) and Menzies *et al.* (1955) indicated that the phosphorus of phosphoric acid was as effective as that of steamed bone meal when the criteria were weight gains and plasma phosphorus levels of cattle maintained under range conditions in Kansas. Further testing of phosphoric acid using the above and other techniques are necessary before a complete evaluation can be made. Recent developments in the radioisotope procedures with farm animals (Hansard, 1956) permit the determination of fecal endogenous phosphorus and the calculation of true digestibility. It seemed desirable, therefore, to determine, using the radioisotope procedures with cattle, the true digestibility of phosphorus in phosphoric acid and to compare this value to the simultaneously determined true digestibility of phosphorus in dicalcium phosphate.

Experimental Procedure

Twelve grade Hereford steers weighing 500 $\pm$ 45 lb. and approximately 14 months of age were divided on the basis of weight, and age into two groups of six per group. One group was fed the ration containing phosphoric acid (ration 1, table 1) and the other group fed the ration containing

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[4] The radiophosphorus was obtained from the Oak Ridge National Laboratories on allocation from the U. S. Atomic Energy Commission. This study was completed under contract No. AT-40-1-Gen-242 between the University of Tennessee and the Atomic Energy Commission.

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dicalcium phosphate (ration 2). Calcium carbonate was added to ration 1 in order to equalize the calcium intakes of all animals and to maintain a calcium to phosphorus ratio of 2.27:1. The daily allowance of each ration was 10 lb., and one-half of it was fed at each of the morning and afternoon feedings. A 14-day preliminary period preceded a 7-day collection period during which time both feces and urine were collected. Both periods were conducted while the animals were in metabolism stalls. The stalls as well as the procedures of handling animals and the excreta were described by Hansard (1951) and Hansard *et al.* (1951). Blood samples were obtained by jugular puncture.

TABLE 1. COMPOSITION OF RATIONS

Item	Ration 1 Phosphoric acid	Ration 2 Dicalcium phosphate
Cottonseed hulls	40.00	40.00
Dried beet pulp	20.00	20.00
Alfalfa meal	10.00	10.00
Urea[a]	1.00	1.00
Sodium chloride	0.50	0.50
Drackett C-l protein	3.00	3.00
Vitamins A and D[b]	0.10	0.10
Starch	12.36	12.41
Cerelose	12.37	12.41
Phosphoric acid	0.48	.....
Calcium carbonate	0.19	.....
Dicalcium phosphate	.....	0.58
Chemical analyses		
Calcium, %	0.509	0.510
Phosphorus, %	0.224	0.224

[a] Courtesy of Dr. D. Green, Allied Chemical and Dye Corporation, New York.
[b] Quadrex type 20, containing 20,000 I.U. Vitamin A and 2,500 USP of D_2 per gram. Courtesy Dr. M. Hochberg, NOPCO Chemical Co., Harrison, New Jersey.

Feed, excreta and blood plasma were analyzed for chemical calcium by the method of Clark and Collip (1925) and for chemical phosphorus by the method of Fiske and Subbarow (1925). Procedures for radiochemistry analyses were described by Comar *et al.* (1951).

At the beginning of the collection period, one-half of the animals in each group received one millicurie of P^{32} orally while the others received the same dosage intravenously and the comparative balance procedure (Hansard, 1952; Comar *et al.*, 1953) and the isotope dilution procedure (Kleiber *et al.*, 1951) were used to determine fecal endogenous phosphorus; the latter method was modified to use a single injection of phosphorus at the initiation of the collection period (Hansard, 1956). The data were analyzed statistically by procedures described by Snedecor (1956).

Results and Discussion

As shown in table 2, treatment differences in all comparisons were statistically insignificant. As the supplements in both ration 1 and 2 provided 60.2% of the phosphorus in rations supplying only 2 gm. of phosphorus per 100 lb. of body weight, there is little doubt that both the phosphoric acid and dicalcium phosphate contained phosphorus that

TABLE 2. DIGESTIBILITY AND BALANCE DATA

Item	Ration 1 Phosphoric acid	Ration 2 Dicalcium phosphate
Phosphorus		
Intake, gm.	10.16	10.16
Feed, gm.	4.04	4.04
Supplement, gain	6.12	6.12
Excretion, gm.	6.65	6.43
Feces, gm.	6.46± .34	6.06± .23
Urine, gm.	0.19± .04	0.37± .15
Fecal endogenous, gm.	4.05± .29	3.54± .21
Retained, % of intake	34.5 ±3.3	36.7 ±1.2
Apparent digestibility, %	36.4 ±3.4	40.4 ±2.3
Endogenous, % of P. intake	39.9 ±2.9	34.8 ±2.1
Endogenous, % of fecal P.	62.7 ±3.5	58.4 ±2.8
True digestibility	76.3 ±3.2	75.2 ±2.5
Calcium		
Intake, gm.	23.09	23.13
Excretion, gm.	16.72	16.98
Feces, gm.	16.54± .51	16.77± .33
Urine, gm.	0.18± .03	0.21±0.16
Retained, % of intake	27.7 ±3.1	26.5 ±2.1
Apparent digestibility, %	28.5 ±3.4	27.4 ±2.5
Various comparisons		
Phosphorus retained, gm.	3.51	3.73
Calcium retained, gm.	6.41	6.11
Calcium to phosphorus	1.83:1	1.64:1

was highly available. Otherwise, based upon endogenous losses of phosphorus noted by Tillman *et al.* (1957) on animals of approximately the same weight and age, the intake of 0.8 gm. of phosphorus per 100 lb. of body weight (supplied by the basal ration) would have exceeded only slightly the fecal endogenous phosphorus, thus would have yielded very low apparent digestion coefficients as well as net retention values. The data indicate that the availability to cattle of phosphorus supplied by reagent grade phosphoric acid was within the same order of magnitude as that furnished by reagent grade dicalcium phosphate, thus by the use of a different technique, confirmed the results of Richardson *et al.* (1955) and Menzies *et al.* (1955).

Summary

Twelve one year old grade Hereford steers weighing approximately 500 lb. each, were used to compare, as phosphorus sources, reagent grade phosphoric acid and dicalcium phosphate. Both phosphorus supplements furnished 60.2% of the phosphorus in rations supplying 2.0 gm. of phosphorus per 100 lb. of liveweight; 39.8% of the phosphorus being supplied by ration ingredients. Criteria were apparent digestibility, net retention, fecal endogenous excretion and true digestibility of phosphorus supplied by the supplements. Treatment differences in all criteria were small and statistically insignificant, indicating the availability of phosphorus supplied by phosphoric acid to be in the same order of magnitude as that supplied by dicalcium phosphate.

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UTILIZATION OF PHYTIN PHOSPHORUS BY SHEEP

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A WIDE variety of plant feedstuffs consumed by farm animals have much of their phosphorus organically bound in a hexaphosphoric acid ester of inositol called phytic acid. It occurs as salts of calcium, magnesium, potassium, etc.; the complex being referred to as phytin. Phosphorus in this form is poorly utilized by non-ruminant animals (Gillis *et al.*, 1953; Chapman *et al.*, 1955; Gillis *et al.*, 1957) but appears to be utilized by ruminant animals (Jordan *et al.*, 1906; Fingerling, 1911; Mathur, 1951). Further studies indicated that rapid hydrolysis took place in the rumen (Reid *et al.*, 1947) and that washed suspensions of rumen microorganisms (Raun *et al.*, 1956) were able to produce phytase, suggesting that the hydrolysis of phytin is independent of the feed. As Reid *et al.* (1947) and Mathur (1951) were able to detect phytin in the undigested material which had passed through the rumen and found that various factors influenced the amount being hydrolyzed within the gastrointestinal tract of ruminants, the need for more research on this question is indicated. It thus seemed desirable to determine with sheep, using the isotope methods outlined by Kleiber *et al.* (1951), Hansard (1952) and Comar *et al.* (1953), the true digestibility of phosphorus supplied by calcium phytate and to compare this value to that determined simultaneously on monocalcium phosphate.

Experimental Procedure

The basal diet, composition of which is shown in table 1, was similar to that used by Ammerman (1956). Ration 1 was formed by feeding each sheep a daily allowance of 680 gm. of the basal diet plus 1.5 gm. of phosphorus supplied by calcium phytate, while ration 2 contained 680 gm. of the basal diet plus 1.5 gm. of phosphorus supplied by a calcium carbonate-monocalcium phosphate mixture. The calcium intakes were equalized between the two rations by the addition of calcium carbonate.

Twelve wether sheep approximately 18 months of age and weighing

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90±10 lb. each were randomly divided into two groups of six animals. Each sheep was fed one-half of his daily allowance of the basal diet and the assigned mineral supplement twice daily during successive 14-day preliminary and 7-day collection periods. Daily collections of feces and urine were made while the sheep were in metabolism stalls described by Briggs and Gallup (1948) and modified for radioisotope procedures by Hansard *et al.* (1951). At the beginning of the collection period, three animals from each group were given concurrently one millicurie of calcium-45 and phosphorus-32 by intravenous injection, while the remaining sheep in each group received the same dosage of each isotope administered

TABLE 1. COMPOSITION OF BASAL RATION

Ingredient	Pounds	Ingredient	Pounds
Alfalfa meal	10.0	Urea [b]	3.5
Cottonseed hulls	10.0	Cottonseed oil	2.9
Woodflock	15.0	Choline chloride	0.1
Corn starch	25.0	Drackett C-1 protein	4.0
Corn sugar	25.0	Vitamins A and D [c]	0.1
Mineral mixture [a]	4.4	Vitamin mixture [d]	+

[a] The percentage composition was as follows: Na_2SO_4, 34.9; $K_8CaH_8O_7 \cdot H_2O$, 33.8; iodized NaCl, 15.4; $CaSO_4 \cdot 2H_2O$, 7.5; $MgCO_3$, 5.83; $FeC_6H_5O_7 \cdot 6H_2O$, 2.36; $MnSO_4 \cdot 6H_2O$, 0.122; $CuSO_4 \cdot 5H_2O$, 0.029; $CoCl_2 \cdot 6H_2O$, 0.24; $ZNCl_2$, 0.020; CaF_2, 0.020; and MoO_3, 0.003.

[b] Feeding grade urea, containing 42.13% nitrogen—courtesy Dr. David Green, Allied Chemical and Dye Corporation, New York, N. Y.

[c] Quadrex, NOPCO Chem. Co., Harrison, New Jersey. Contained 20,000 I.U. Vitamin A and 2,500 USP units of Vitamin D_3 per gram. Courtesy Dr. M. Hochberg.

[d] vitamins added in mg. per lb. of ration: Ascorbic acid, 14.0; biotin, 0.03; calcium pantothenate, 27.0; folic acid, 0.2; inositol, 90.0; niacin, 18.0; p. aminobenzoic acid, 10.0; pyridoxine HCl, 2.25; riboflavin, 4.5; thiamin-HCl, 2.25; 2-methyl-1, 4-naphthoquione, 0.9; and vitamin B_{12}, 0.015.

orally. Calculations of fecal endogenous calcium and phosphorus were made by the isotope dilution method described by Kleiber *et al.* (1951) which was modified to allow a single injection of the isotope (Hansard, 1956) and by the comparative balance method as described by Hansard (1952) and Comar *et al.* (1953). Blood samples, obtained from all animals by jugular puncture, were taken at frequent intervals during the collection period for determination of both chemical and radiochemical calcium and phosphorus. Calcium was determined by the method of Clark and Collip (1925) and modified for radiocalcium studies by Comar *et al.* (1951). Separation of radiocalcium and radiophosphorus was made by using an aluminum absorber having a mass of 55 mg. per cm^2 to absorb the weaker beta emissions of radiocalcium. Details of the procedure and the method used for self absorption corrections for radiocalcium were described by Comar *et al.* (1951). Inorganic phosphorus analyses were made by the method of Fiske and Subbarow (1925). Radioassay of both isotopes was performed by using a thin mica window Geiger-Mueller counter connected to a scaler. Phytin phosphorus analyses of both feed and feces were made by the

method of Common (1939). Statistical analyses of the data were by the analysis of variance method of Snedecor (1956).

A modification of the phytin-phosphorus procedure of Common (1939) was used to determine *in vitro* the exchange of phosphorus of calcium phytate with phosphorus-32. Briefly, the procedure was as follows: Ten mg. of calcium phytate were weighed into a 40 ml. centrifuge tube. To this was added 10 ml. of distilled water and 1 ml. of P-32 having a count of 2.4×10^5 counts per minute. Dilute sodium hydroxide was added to obtain the desired pH and the mixture with constant shaking was incubated at 37° C. for 24 hours. After incubation was completed a few drops of 0.5 N HCl and 10 ml. of a $FeCl_3$-HCl solution were added; the latter solution contained 1.0 gm. of iron per liter dissolved in normal HCl. The mixture was then heated on a water bath for 15 minutes and cooled and centrifuged for 10 minutes at 3000 rpm. The supernatant was decanted into a 50 ml. volumetric flask. The precipitate was broken up, using a fine stream of water, and then 5 ml. of 0.5 N HCl were added with the solution being recentrifuged and decanted as before; this being repeated 4 times. To the precipitate were added 5 ml. of hot water and 5 ml. of 2% NaOH solution and the mixture was heated on a water bath for 15 minutes. The 10 ml. of solution was then transferred to a petri dish for wet counting in a Geiger-Mueller counter. The supernatant was made up to volume and a 10 ml. aliquot was transferred to a petri dish for wet counting. For relative counting a standard was diluted to 10 ml. and transferred to the same type of petri dish. Using this procedure, the degree of exchange was determined at a pH of 1.0, 3.0 and 6.5.

Results and Discussion

The results obtained in the digestibility and balance trials are shown in table 2. When the criteria were apparent digestibilities, endogenous fecal excretions, true digestibilities, and net retentions of calcium and phosphorus, differences between values reported for calcium phytate and monocalcium phosphate were small and insignificant statistically. If monocalcium phosphate is a highly available source of phosphorus for ruminants (Morrison, 1957; Plumlee and Beeson, 1953) the calcium phytate used in this study is likewise highly available. When the true digestibility of the phosphorus supplied by monocalcium phosphate is assigned a value of 100, the relative value of calcium phytate becomes 90. It is interesting to note (also shown in table 2) that approximately 92% of the phytin phosphorus was hydrolyzed and thus would become available for use by ruminant microorganism (Raun *et al.*, 1956) and the host animal (Jordon, 1906). Thus, the results obtained in the present experiment by the use of isotope techniques confirm those of Jordon *et al.* (1906); Fingerling (1911); and Reid *et al.* (1947), who reported that ruminant animals were able to utilize phytin phosphorus.

Figure 1 shows, graphically, the accumulation of orally- and intravenously-administered phosphorus-32 in the feces. At the end of the 7-day collection period, both groups had excreted approximately 61% of the

TABLE 2. DIGESTIBILITY, ENDOGENOUS EXCRETION, AND DAILY BALANCES OF CALCIUM AND PHOSPHORUS AND THE HYDROLYSIS OF PHYTIN

Item	Ration 1 Calcium phytate	Ration 2 Monocalcium phosphate
Phosphorus		
Intake, gm.	2.14	2.14
Feed, gm.	0.64	0.64
Supplement, gm.	1.50	1.50
Excretion, gm.	2.49(0.02) [a]	2.51(0.16)
Feces, gm.	2.41(0.02)	2.41(0.16)
Urine, gm.	0.08(0.001)	0.10(0.002)
Fecal endogenous, gm.	1.61(0.02)	1.76(0.21)
Retained, % of intake	−16.4 (2.9)	−17.3 (8.3)
Apparent digestibility, %	−12.6 (2.9)	−12.6 (8.3)
Endogenous, % of P intake	75.7	82.2
Endogenous, % of fecal P.	66.8	73.0
True digestibility, %	62.6 (4.4)	69.6 (4.6)
Calcium		
Intake, gm.	3.37	3.37
Feed, gm.	1.77	1.77
Supplement, gm.	1.60	1.60
Excretion, gm.	4.29(0.03)	4.25(0.31)
Feces, gm.	4.17(0.03)	4.08(0.33)
Urine, gm.	0.12(0.002)	0.17(0.031)
Fecal endogenous, gm.	2.54(0.21)	2.36(0.19)
Retained, % of intake	−27.3 (1.6)	−26.1 (2.8)
Apparent digestibility, %	−23.7 (1.7)	−21.1 (2.9)
Endogenous, % of Ca. intake	75.4	70.0
Endogenous, % of fecal Ca.	60.9	57.8
True digestibility, %	51.6 (6.6)	49.0 (7.3)
Phytin phosphorus		
Intake, gm.	1.83	
Feed, gm.	0.33	
Calcium phytate, gm.	1.50	
Feces, gm.	0.15	
Digestibility, %	91.8(0.7)	

[a] Standard deviation of mean.

orally-administered dose, indicating there was no influence of treatment upon this phenomena. In the earlier hours after oral dosing, however, there were treatment differences in the rate of accumulation of phosphorus-32 in the feces. Twelve hours after dosing, the feces of the control animals contained over twice as much ($P<0.05$) phosphorus-32 as those from the

animals fed phytate and also in the following 12-hour period, the control group continued to excrete more fecal phosphorus-32 ($P<0.05$) than those fed phytate. During the first 24 hours the control group also excreted 20% more stable phosphorus; however, if an allowance for increased fecal output were applied to the control group, it was found that this group still excreted significantly ($P<0.05$) more phosphorus-32 than those receiving phytate. In the following 24-hour period (ending 48 hours after dosing) the trend was reversed and, during this period, the group receiving phytate excreted 1.7 times ($P<0.05$) more fecal phosphorus-32 than the control animal, and then continued to excrete slightly more fecal

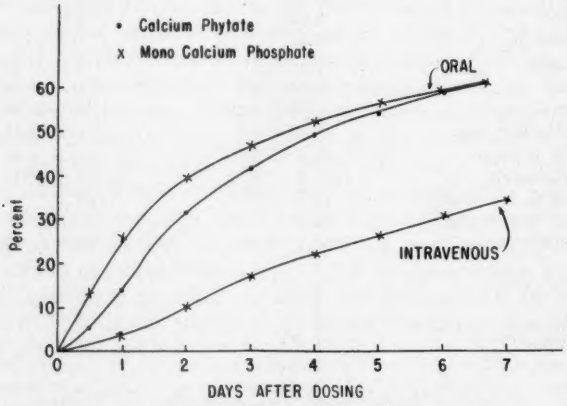

Figure 1. Accumulation of administered P-32 in feces.

phosphorus-32 during each subsequent 24-hour period until, at the end of the experiment, the total fecal phosphorus-32 excretions were the same for both groups. Thus, the data could be interpreted to mean that, in some way, the orally-administered phosphorus-32 was being held temporarily in the rumen, thus, delaying its passage along the gastrointestinal tract. An attractive theory along these lines involves the idea of exchange of the inorganic phopshorus-32 with the phosphorus in the phytin molecule and that the phosphorus-32 so combined would be released from this combination and become available for utilization by the animal when the phytin molecule was hydrolyzed by the enzyme, phytase. If this were true, the greatest degree of exchange would be expected to occur immdiately after dosing; the animals were fed approximately 2 hours prior to dosing. The results of Reid *et al.* (1947) which indicated that complete hydrolysis of phytin took place within 8 hours after it entered the rumen adds support to this idea. Also, as shown in figure 2, blood samples taken at 1 and 5 hours after oral administration of the isotope indicated a higher concentration

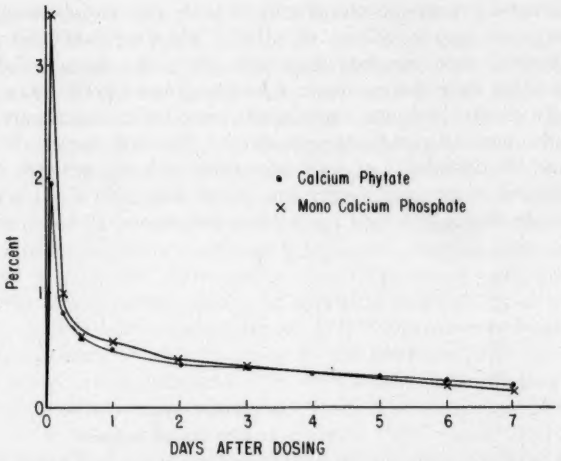

Figure 2. Percent of administered phosphorus-32 in the blood plasma following oral administration.

of phosphorus-32 ($P<0.01$ and $P<0.05$, respectively) in the plasma of the control animals. In all subsequent analyses (6, 12, 24, 48, 168 hours) there were small and insignificant treatment differences. Disregarding factors causing more rapid movement of phosphorus-32 out of the plasma, the data would indicate that at these times (1 and 5 hours) there had been more rapid absorption of the nuclide from the gastrointestinal tracts of the animals fed monocalcium phosphate. Later, as the phytin molecule was hydrolyzed, the phosphorus-32 of the animals fed calcium phytase was absorbed and 6 hours after dosing, the concentration of phosphorus-32 in the plasma of both groups was equalized. Even though the results of the *in vitro* exchange studies, shown in table 3, did not reveal the amount

TABLE 3. RESULTS OF *IN VITRO* STUDIES REGARDING THE POSSIBILITY OF PHOSPHATE EXCHANGES BETWEEN PHYTATE AND INORGANIC PHOSPHORUS-32

pH	Radioactivity added as $Na_2HP^{32}O_4$	Calcium phytate added	Percent radioactivity in		Recovery of radioactivity
			Filtrate[a]	Precipitate[b]	
	CPM[a]	mg.	%	%	%
1.0	2.4×10^5	10	95.0±0.3	5.0±0.3	99.3±0.4
3.0	2.4×10^5	10	95.1±0.9	4.9±0.9	99.1±1.3
6.5	2.4×10^5	10	96.9±0.6	3.1±0.6	97.4±0.8

[a] CPM=counts per minute.
[b] Contained inorganic phosphorus.
[c] Contained phytin phosphorus.

of exchange of inorganic phosphorus-32 with the phosphorus of calcium phytate as reported by Gillis *et al.* (1957), who used a different procedure, it is apparent that some exchange took place. We do not wish to draw inference that these observations can be extrapolated to the living organism. Certainly, precise biological experiments using living animals are necessary before the question of exchange is answered. The data suggest, in a qualitative way, the probability of some phosphate exchange between the phytin molecule and an inorganic source and, in this way, offer a possible explanation for the observation that for the first and second 12-hour periods after

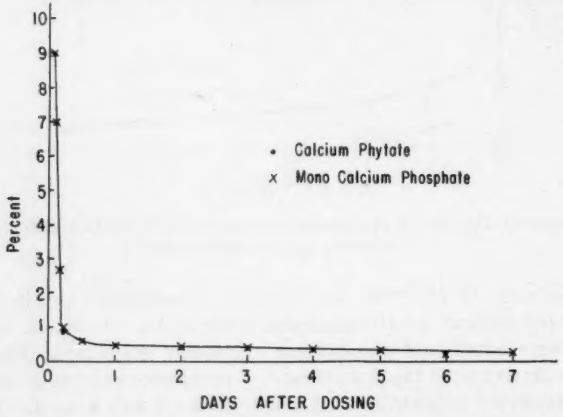

Figure 3. Percent of administered phosphorus-32 in the blood plasma following intravenous administration.

oral administration of phosphorus-32, there was less of this nuclide in the feces of animals fed calcium phytate.

A discrepancy ($P<0.10$) was noted between the results for fecal endogenous phosphorus excretions as calculated by the comparative balance and isotope dilution procedures. The isotope dilution procedure yielded higher values with both rations. This lack of agreement between the two techniques, as applied to phosphorus-32 uptake and excretion data in the present study, warrants further study, particularly in view of the findings of previous workers showing satisfactory agreement between the two procedures (Comar *et al.*, 1953; Shroder, 1957). This is made even more necessary by the fact that in the present study fairly close agreement was obtained for the calcium data calculated by the two procedures. In a comparative study of the two procedures, Comar *et al.* (1953) state that the primary assumption in the comparative balance method is that all of the dietary calcium, feed calcium plus the labelled calcium given as soluble calcium chloride, are absorbed to an equal degree. An alternative assump-

tion, suggested by Thompson (1957), is that the orally-administered radioactive element and the stable form in the feed must reach complete equilibrium before independent absorption of the active element takes place. If such an equilibrium was established, there could be little doubt that this method would give an accurate estimate of true digestibility of a dietary element, but if not, then much of the data would apply to the absorption of the orally-administered radioactive element, and not to the dietary form. There is little reason to suppose that cations such as calcium are present in feeds as non-exchangeable ions; thus, an equilibrium of feed calcium with the orally-administered calcium-45 would be expected to be established very quickly. However, in view of the many phosphorus-containing organic compounds present in biological materials, it seems likely that some of the dietary phosphorus exists in forms not readily exchangeable with administered phosphorus-32. If this were true, the true digestibility of phosphorus calculated by the comparative balance appertains to the true digestibility of the exchangebale fraction of the dietary phosphorus and not to the dietary phosphorus as a whole. More research work should be conducted to find out if orally-administered phosphorus-32 is exchangeable with the various organic phosphorus compounds before the comparative balance technique is used in all phosphorus availability studies.

Summary

Twelve wether sheep approximately 18 months old and weighing approximately 90 lb. each were used to compare, as phosphorus sources, calcium phytate and monocalcium phosphate. Both phosphorus supplements supplied 70% of the phosphorus in rations supplying 2.38 gm. of phosphorus per 100 lb. of liveweight. Criteria of response were apparent digestibilities, net retention, fecal endogenous excretions, and true digestibilities of dietary calcium and phosphorus. Treatment differences in all criteria were small and statistically insignificant, indicating the availabilities of both calcium and phosphorus contained in calcium phytate were in the same order of magnitude as those supplied by monocalcium phosphate. Methodology as applied to radioisotope procedures for measuring fecal endogenous excretion of calcium and phosphorus was discussed.

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EVALUATION BY LAMB AND STEER FEEDING TESTS OF ALFALFA HAY OF VARYING PHOSPHORUS CONTENT

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University of California

PHOSPHORUS deficiency in soils and the forage produced therefrom and aphosphorosis in grazing animals has been widely recognized in many parts of the world. Theiler and Green (1932) and Mitchell and McClure (1937) have carefully reviewed the problem of phosphorus needs of livestock. The use of phosphate fertilizers as a means of correcting soils low in phosphorus is a common practice. Heinemann *et al.* (1957) have recently summarized the literature on the effects of fertilization with phosphate on the feeding value of the forage. These authors reported improved performance of rabbits fed fertilized hay compared to unfertilized hay of a lower phophorus content.

Early fertilizer trials in the Imperial Valley of California demonstrate marked yield increases when alfalfa was fertilized with phosphorus-containing fertilizers. Farmers in the area generally believe that the feeding value of the alfalfa is improved as well as the yield as a result of the application of fertilizer. This paper reports a series of tests conducted at the Imperial Valley Field Station and the Davis Campus of the University of California to explore differences in feeding value of alfalfa produced on soils with various fertilization history.

Experimental

Chemical analyses, digestibility trials, balance trials and feedlot feeding trials were used to test the various feeds. To maintain continuity the various trials are reported chronologically, however, certain techniques were common to all the work and will be reported first to avoid repetition. In feeding trials all animal weights were obtained after 24 hours without feed and 12 hours without water. Except in the second cattle feeding test, all animals were randomly assigned to treatments. For the digestion and balance trials a Latin-square design was employed. Seven-day preliminary and 7-day collection periods were used. Phosphorus determinations in all material were based on the method of Fiske and Subbarow (1925). Lignin was determined according to the method of Ellis *et al.* (1946). Other chemical analyses were by A.O.A.C. method (1950). Blood samples collected from

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[4] The authors gratefully acknowledge the technical assistance of George N. Hill and T. S. Inouye.

animals on test at the Imperial Valley Field Station were refrigerated, the serum collected and shipped by air to Davis for analysis.

First Cattle Feeding Test. The alfalfa for the initial trial was obtained from an area supervised by Imperial County Agricultural Extension Service personnel. One irrigation check had not been fertilized for seven years. Phosphate fertilizer applied the fall of the sixth year—to a small portion of the check—gave yield increases from 30% to 60% during the spring months of the seventh and eighth years. The hay obtained from the non-fertilized remainder of the check was used as the unfertilized hay in the

TABLE 1. FIRST CATTLE FEEDING TEST WITH FERTILIZED AND UNFERTILIZED ALFALFA HAY

	Fertilized	Unfertilized
Phosphorus content of hay, %	0.24	0.19
Length of trial, days	96	96
No. of steers	5	5
Average initial weight, lb.	391	392
Average final weight, lb.	564	541
Average daily gain, lb.	1.80	1.55
Average daily feed intake, lb.	14.86	14.51
Feed per 100 lb. gain	826	936
Blood serum phosphorus (mg. per 100 ml. serum)		
Initial	8.07	7.53
Final	8.80	7.46

first test. The fertilized hay came from the adjoining check which had been fertilized annually and had received 175 lb. of P_2O_5 per acre as superphosphate the fall before the hay used in this test was grown.

After cutting, the hay was sun-cured and baled. Each of the two lots of hay was fed to five Hereford steers. The animals received no other feed. Salt and water were always available in the feedlot. The results are summarized in table 1. Although the average daily gain appeared to favor the fertilized hay the differences were not significant statistically. Other differences between lots were considered inconclusive.

Second Cattle Feeding Test. In this test, the feeding value of alfalfa hay grown on two different soils was compared. One site, on the Field Station, had received annual applications of phosphate sufficient to maintain yields. This soil has been under cultivation and irrigation for many years. The second site, although only a few miles distant, was desert sandy soil only very recently brought under sprinkler irrigation. The soil had not been fertilized. Analyses of the alfalfa grown indicated that the soil was acutely deficient in phosphorus. It was not possible to obtain fertilized hay from this second site. The unfertilized hay graded U.S. No. 1, extra leafy while the fertilized hay graded U.S. No. 2, leafy.

TABLE 2. COMPOSITION OF HAYS USED IN SECOND STEER FEEDING TRIAL
(DRY BASIS)

	Fertilized	Unfertilized
Phosphorus, %	0.23	0.10
Sand and silica, %	.66	8.34
Crude fiber	21.5	13.3
Organic matter, (% of dry matter)	89.1	74.2
Ash, %	10.9	25.8
Crude protein, %	21.5	14.4
Chlorine, %	1.30	.24
Calcium, %	1.57	5.37
Sulfur, %	.33	.24
Magnesium, %	.27	.42
Potassium, %	3.1	1.9
Sodium, %	0.39	0.19

The steers used in the first test were again used after regrouping on the basis of gains made in the first test. Again the steers were fed unchopped, baled hay ad libitum with water and salt available.

The composition of the two lots of hay is shown in table 2. The phosphorus of the unfertilized hay was very low and in the range generally considered deficient, but there were many other differences in the composition of the two hays. The unfertilized hay contained a large amount of sand which had been picked up in the haying process.

The results of the feeding trial are shown in table 3. From the beginning of the test the animals practically refused to eat the hay from the unfertilized area with the average daily feed intake a little more than half of that of the control lot. The gains on the unfertilized hay were uniformly low and the gains on the fertilized hay uniformly high. The blood serum phosphorus dropped in all steers fed the unfertilized hay.

TABLE 3. SECOND CATTLE FEEDING TEST WITH FERTILIZED AND UNFERTILIZED ALFALFA HAY

	Fertilized	Unfertilized
Length of trial, days	91	91
Number of steers	5	5
Average initial weight, lb.	543	558
Average final weight, lb.	743	606
Average daily gain, lb.	2.19	0.53
Average daily feed, lb.	20.6	11.2
Feed per 100 lb. gain, lb.	940	2130
Blood serum phosphorus (mg. per 100 ml. serum)		
Start of test	8.13	8.15
End of test	7.32	4.79

Third Cattle Feeding Test. A third cattle test was then conducted with the same animals. Using the same hays as in the second test, sufficient disodium phosphate was added to the unfertilized hay to bring the phosphorus content up to that of the fertilized hay. This test ran for only 27 days. The addition of the disodium phosphate did not increase the palatability of the unfertilized hay and the feed intake and gains were about the same as during the second test. The addition of the disodium phosphate did result in an increase in the blood phosphorus of the cattle fed the unfertilized hay from 4.79 initially to 8.28 mg. at the end of the 27-day period.

First Sheep Feeding Test. As the results of the trials did not clearly show whether phosphorus was the primary difference in the two hays, an attempt was made to establish fertilizer tests in the area where the unfertilized hay was produced. Unfortunately, the land had changed ownership and had all been fertilized so this approach to the problem could not be used. With a limited amount of the original unfertilized hay on hand, a sheep trial was conducted to study the problem further. In the first sheep test the hays which had been used in the second steer feeding test—that is, a high phosphorus hay produced at the Field Station from fertilized soil and the low phosphorus hay produced on the virgin desert soil—were ground and pelleted for the test. In addition, the low phosphorus unfertilized hay was supplemented with disodium phosphate to bring the phosphorus content up to the level found in the fertilized hay. An additional two lots were fed hay grown at the Imperial Valley Field Station on a plot which had previously shown a phosphorus fertilizer response but had not been fertilized for five years. One lot was fed unsupplemented pelleted hay containing 0.15% phosphorus. The other lot was fed the same hay but with sufficient disodium phosphate added to bring the phosphorus level up to the fertilized Station hay (23% P). As the hay for three of the lots was two years old when the pellets were made, stabilized vitamin A was added to the pellets for all lots to preclude any possibility of a vitamin A deficiency being encountered.

The composition of the pellets fed in this first sheep test are shown in table 4. In addition to the differences in phosphorus content the unfertilized hay from the desert soil was much higher in ash, calcium and sand in silica and lower in crude protein, crude fiber, chlorine and potassium. (See also table 2). The results of the lamb feeding trial are shown in table 5. There was essentially no difference in the performance of the lambs on the two Station hays even with disodium phosphate added to the hay which was somewhat lower in phosphorus. This appears to indicate that under the conditions of this trial 0.15% phosphorus was adequate for the lambs. As in the steer feeding test the unfertilized desert hay was unpalatable even in pelleted form. The addition of disodium phosphate increased its palatability only slightly as judged by the average daily feed intake. Lambs fed this hay used their feed more efficiently than the lambs fed the same

TABLE 4. COMPOSITION OF FEEDS USED IN FIRST SHEEP FEEDING TEST AND DIGESTION AND BALANCE TRIAL
(DRY BASIS)

	Fertilized station hay	Unfertilized station hay	Unfertilized station hay + phosphorus	Unfertilized desert hay	Unfertilized desert hay + phosphorus
P content of hay, %	0.23	0.15	.22	0.10	0.23
Crude protein, %	21.5	18.4	17.2	14.4	14.5
Crude fiber, %	21.5	22.6	22.9	13.3	13.8
Nitrogen-free extract, %	44.6	47.0	47.7	45.4	45.5
Ether extract, %	1.5	2.0	2.0	1.1	1.1
Organic matter, %	89.1	90.0	89.8	74.2	74.9
Ash, %	10.9	10.0	10.2	25.8	25.1
Sand and silica, %	0.66	1.03	0.97	8.34	7.08
Calcium, %	1.57	1.66	1.57	5.37	5.26

hay without phosphorus. The average daily gain was significantly improved by the additional phosphorus, yet both lots receiving the desert hay still gained very significantly less than the lambs fed the various forms of Station hay. The feed required per 100 lb. of gain is markedly in favor of the lambs fed the Station hays, however, when the feed efficiency is expressed on the basis of the organic matter required per 100 lb. of gain the

TABLE 5. RESULTS OF FIRST SHEEP FEEDING TEST
(112 DAYS)

	Fertilized station hay	Unfertilized station hay	Unfertilized station hay + phosphorus	Unfertilized desert hay	Unfertilized desert hay + phosphorus
Number of lambs	9	10	10	9	10
Av. initial weight, lb.	64.9	62.7	62.0	63.2	63.0
Av. final weight, lb.	114.6	105.5	106.3	76.8	87.7
Av. daily gain, lb.	.44	.38	.40	.12[a]	.22[b]
Av. daily feed, lb.	3.46	3.40	3.48	2.25	2.33
Feed per 100 lb. gain, lb.	781	889	880	1861	1058
Organic matter per 100 lb. gain, lb.	696	800	790	1380	792
Salt consumption per lamb, lb.	1.0	2.0	0.6	4.9	3.0
Blood serum phosphorus (mg. per 100 ml. serum)					
Initial	6.48	6.45	6.09	6.20	5.67
Final	6.11	6.67	7.11	4.41[c]	7.86

[a] Gain significantly less ($P<.05$) than unfertilized desert hay plus phosphorus.
[b] Gain significantly less ($P<.01$) than on station hays.
[c] Serum phosphorus significantly lower ($P<.01$) than other lots.

desert hay with the supplemental phosphorus compares favorably with the efficiency of the Station hay. The final blood serum phosphorus values for the lot fed the unfertilized desert hay were significantly lower than the values for the other lots. The addition of the disodium phosphate to this hay prevented this drop in blood phosphorus as it had corrected the lowered blood phophorus in the third steer feeding trial.

As in the steer feeding trial on the desert hay, it had appeared that the steers on the desert hay were consuming more salt, the salt consumption of the various lots in this trial was measured. The salt consumption per lamb was markedly high in the lot fed the unsupplemented-unfertilized desert hay. The addition of the disodium phosphate reduced the salt intake appreciably but it was still higher than in the lots fed the Station hay. It is interesting to note that the addition of disodium phosphate to the Station hay also reduced the salt intake compared to the lambs fed the same hay without the additional disodium phosphate. Perhaps the additional sodium in the disodium phosphate decreased the salt need of the sheep.

To study further the differences in the feeds used in the feeding trial, pellets of each lot of feed were shipped to Davis where digestion and balance trials were conducted using wethers approximately one year of age. Feeds were fed on an equal organic matter intake estimated to furnish 110% of maintenance. All sheep completed the trial on the Station hays without incident, but trouble was encountered in getting the sheep to eat a constant intake of the desert hay either with or without the added phosphorus. After repeated efforts two sheep finished on the unfertilized desert hay and three finished on the same hay plus the disodium phosphate. The results of the trial are shown in table 6. The results of the digestion trial gave no readily apparent answer as to why the three Station hays gave so much better gains. The lower total digestible nutrients and the lower digestible protein content reflect the dilution of the high ash content rather than an impaired digestibility of any of the nutrients. The nitrogen retention varied so widely within treatments that there was no significant difference between rations. The phosphorus retention by the lambs fed the desert hay plus sodium phosphate was significantly higher than the retention by the lambs fed the other rations. The calcium retention was very significantly higher by the lambs fed the desert unfertilized hay both with and without the supplemental phosphorus.

Second Sheep Feeding Test. As the results of the above trials were not conclusive another attempt was made to obtain hay from the area where the original desert hay was grown. In the meantime two growing seasons had passed and the ownership of the land had changed. The new owner was most cooperative and set aside approximately 17 acres for experimental use. He understood that 100 lb. of liquid phosphate (52% P) had been applied to the area two seasons before and knew that 200 lb. had been applied the previous growing season.

To obtain forage from this area of varying phosphorus contents, the area

was divided into 3 plots. About half the area was left unfertilized, one-fourth was fertilized with 600 lb. of treble super phosphate per acre and the remaining one-fourth with 150 lb. of treble super phosphate. The third cutting of alfalfa from the area was used for the second sheep feeding trial. All plots were cut at the same time when the alfalfa was at about the one-tenth bloom stage. The hay was raked carefully so as to pick up as little sand as possible. The hay was then baled, ground and pelleted. After

TABLE 6. RESULTS OF DIGESTION AND BALANCE TRIAL WITH FEEDS USED IN FIRST LAMB FEEDING TRIAL

	Fertilized station hay	Unfertilized station hay	Unfertilized station hay + phosphorus	Unfertilized desert hay	Unfertilized desert hay + phosphorus
Digestion coefficients, %					
Organic matter	65.9	65.8	64.0	73.2	69.5
Crude protein	74.7	74.1	71.3	73.9	71.7
Crude fiber	41.8	43.5	40.0	48.2	38.8
Nitrogen-free extract	74.5	75.4	74.8	80.9	79.3
Ether extract	32.1	18.9	17.8	49.9	22.4
Total digestible nutrients (dry basis)	59.4	59.7	58.1	55.0	52.4
Digestible protein, %	16.1	13.6	12.3	10.6	10.4
Balance trial					
Retention in grams per day					
Nitrogen	2.61	2.05	1.00	1.16	2.34
Phosphorus	0.21	0.03	0.08	0.14	0.62*
Calcium	—0.17	0.32	0.02	6.88**	7.06**

* Significantly higher phosphorus retention than other lots ($P<.05$).
** V. significantly higher calcium retention than other lots ($P<.01$).

grinding, the hay from the unfertilized area was divided into two lots. One lot received added disodium phosphate to bring the phophorus content up to that of the hay grown on the plot receiving the largest amount of fertilizer. The composition of the pelleted feed is shown in table 7. The phosphorus content of this unfertilized hay was not as low as that obtained for the earlier experiments. Evidently the fertilizer applied the two seasons previously carried over enough to correct the very low level found in the original hay. The lower level of fertilizer only improved the phosphorus content slightly while the heavy application of fertilizer markedly increased the phosphorus content. In other respects the composition of the hay produced on the three plots varied only slightly. The calcium and sand and silica were much lower than in the hay from the same area used in the earlier tests.

The pelleted hays were fed to feeder lambs with the results shown in table 8. There was little, if any, difference in the response of the lambs to

TABLE 7. COMPOSITION OF FEEDS USED IN SECOND SHEEP FEEDING TEST AND DIGESTION AND BALANCE TRIALS
(DRY BASIS)

	Heavily** fertilized desert hay	Lightly* fertilized desert hay	Unfertilized desert hay	Unfertilized desert hay + phosphorus
Phosphorus content, %	0.22	0.17	0.15	0.25
Crude protein, %	16.0	14.7	15.0	14.9
Crude fiber, %	26.0	27.8	25.8	26.4
Nitrogen-free extract, %	46.8	47.1	48.0	48.3
Ether extract, %	.9	1.0	1.0	1.1
Organic matter, %	89.7	90.6	89.8	90.7
Ash, %	10.3	9.4	10.2	9.3
Sand and silica, %	.7	.9	1.2	1.3
Calcium, %	1.4	1.3	1.2	1.1
Lignin, %	9.0	10.0	9.6	9.3

* 150 lb. treble super phosphate per acre.
** 600 lb. treble super phosphate per acre.

the four lots of feed. Although the data show a slight advantage in favor of the unfertilized hay with the added phosphorus, the difference was not significant statistically. Much of the difference in gain could be accounted for by one lamb which gained poorly.

These four lots of feed were also tested with a digestion and balance trial similar to that conducted on the feeds from the first lamb feeding trial. The results are shown in table 9. The digestion data showed little difference between the hays. The digestion coefficients for all nutrients were slightly higher for the hay from the area that was heavily fertilized but the differ-

TABLE 8. RESULTS OF SECOND SHEEP FEEDING TEST
NOVEMBER 16 TO JANUARY 18, 1955
(64 DAYS)

	Heavily fertilized desert hay	Lightly fertilized desert hay	Unfertilized desert hay	Unfertilized desert hay + phosphorus
Number of lambs	10	9	10	10
Average initial weight, lb.	76.5	73.6	73.7	72.5
Average final weight, lb.	100.7	99.9	97.0	101.2
Average daily gain, lb.	.38	.41	.36	.45
Average daily feed, lb.	4.22	4.31	3.95	4.23
Feed per 100 lb. gain, lb.	1117	1046	1084	944
Blood serum phosphorus (mg. per 100 ml. serum)				
Initial	7.5	7.4	7.3	7.5
Final	7.5	7.2	7.7	7.5

ences were not significant statistically. As in the earlier balance trials there was no difference in the nitrogen retention between the different treatments. The phosphorus retention was again high in the lambs fed the feed supplemented with disodium phosphate. In this trial the retention by the lambs fed the heavily fertilized hay was also high, but very low in the lambs fed the hay from the plot that received a light application of fertilizer. The calcium retention was very different from that found in the previous test. In the first test large amounts of calcium were retained from the unfer-

TABLE 9. RESULTS OF DIGESTION AND BALANCE TRIAL WITH FEEDS USED IN SECOND LAMB FEEDING TRIAL

	Heavily fertilized desert hay	Lightly fertilized desert hay	Unfertilized desert hay	Unfertilized desert hay + phosphorus
Digestion coefficients, %				
Organic matter	62.9	58.5	59.5	59.9
Crude protein	73.2	70.0	70.8	69.6
Crude fiber	35.8	30.1	30.7	31.4
Nitrogen-free extract	76.1	72.1	73.1	75.8
Ether extract	19.8	−7.0	27.9	−10.0
Total digestible nutrients (dry basis)	56.6	52.7	53.6	55.3
Balance trial (Retention in grams per day)				
Nitrogen	3.05	2.18	1.09	1.86
Phosphorus *	0.53	0.01	0.16	0.51
Calcium **	1.60	0.10	−0.89	−1.48

* LSD for phosphorus retention $P<.05=0.11$ $P<.01=0.17$.
** LSD for calcium retention $P<.05=1.26$ $P<.01=1.91$.

tilized hay with and without phosphorus added but in the second trial the unfertilized hay both with and without added phosphorus had negative balances.

Discussion

As pointed out in a previous publication (Rendig and Weir, 1957), evaluation of forages from soils receiving differing treatments involves many complications. While phosphorus was the most obvious difference in the composition of the hays involved in this series of trials, other constituents of the feed also varied. Probably the most serious contaminant was the high sand and silica found in the unfertilized desert hay used in the second steer feeding trial and the first lamb feeding trial. The extreme unpalatability of this hay cannot be explained with the information available. The addition of disodium phosphate to this hay before pelleting improved its palatability only slightly. The increased gains, feed efficiency

and blood serum phosphorus levels resulting from the addition of the disodium phosphate clearly indicate that phosphorus was at least one of the reasons for the poor animal response to the original hay.

The lack of animal response to the addition of disodium phosphate to the unfertilized Station hay and the desert hay in the second lamb feeding trial appears to indicate that 0.15% phosphorus is not limiting for growing lambs. The response to the disodium in the original desert hay (first lamb feeding trial) suggests that 0.10% phosphorus is limiting. The daily intake of phosphorus on the desert hay was much lower because of the lower feed intake. In the first lamb feeding trials the lambs on the feed containing 0.15% phosphorus ate 3.4 lb. of feed per day to get approximately 2.31 gm. P daily or about 2.75 gm. per 100 lb. body weight. In the second lamb feeding trial the lambs ate 3.95 lb. of feed per day to get 2.67 gm. P daily or 4.09 gm. per 100 lb. live weight. On the unfertilized desert hay in the first trial when the lambs only consumed 2.25 lb. of the hay containing 0.10% phosphorus their daily intake was only 1.02 gm. or 1.46 gm. per 100 lb. body weight. Beeson *et al.* (1944) state that 0.15% is the minimum phosphorus level for fattening lambs and that 2.17 gm. daily per 100 lb. live weight will meet their phosphorus requirement. It is doubtful if the depressed intake was due to a phosphorus deficiency in the animals, however, because both the cattle and sheep showed an aversion to this hay immediately rather than after being fed on it long enough to develop a deficiency.

The digestion trial data did not indicate any difference in the digestibility of the various nutrients. As a Latin-square design was used in these tests no deficiency symptoms could be expected to appear during the short time the sheep were on each ration. The lower feed efficiency (expressed on an organic matter basis) of the sheep fed the unfertilized desert hay in the first lamb feeding trial may indicate a deficiency of phosphorus interfered with feed utilization. This would be in accord with the findings of Beeson *et al.* (1944).

Summary

Feeding trials with both sheep and steers and digestion and balance tests were conducted on alfalfa hay of varying phosphorus content to study its nutritive value. Hay produced on virgin sandy desert soil which contained 0.10% phosphorus was unpalatable and produced poor animal gains, feed utilization and depressed serum phosphorus content. The addition of disodium phosphate to this hay improved animal gains, feed utilization and increased the blood serum phosphorus. Hay containing 0.15% phosphorus was apparently equal in feeding value to hay containing greater amounts of phosphorus. Digestion trials on the various hays did not reveal any significant differences in the digestibility of the various nutrients. Balance trials showed no difference in nitrogen retention and a variable calcium balance. An increased retention of phosphorus was obtained by adding diso-

dium phosphate to a low phosphorus hay in one test, in another, the addition of disodium phosphate to unfertilized hay resulted in increasing the phosphorus retention up to that of heavily fertilized hay.

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THE EFFECT OF CERTAIN FEED ADDITIVES ON THE TISSUES OF FATTENING BEEF CATTLE[1]

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THE use of feed additives in the rations of fattening beef cattle is increasing. Approximately ⅔ of the cattle fattened in the United States are now receiving stilbestrol or other estrogens during the fattening period (Anonymous, 1957). Other cattle are fed antibiotics alone, and in combination with stilbestrol. In addition, the use of animal fat in livestock rations has been accellerating as the demand for fat for soap production has decreased. The purpose of this experiment was to determine the effect of chlortetracycline, diethylstilbestrol and graded levels of animal fat on the composition of the blood, liver and carcass of fattening beef cattle.

Experimental Procedure

Forty-eight steers approximately two years of age were allotted at random to treatments in a 2x2x3 factorial designed experiment. The experimental treatments were: 2 levels of chlortetracycline, 0 and 70 mg. daily; 2 levels of stilbestrol, 0 and 10 mg. daily; and 3 levels of added animal fat, predominantly inedible beef tallow, 0, 5, and 10% of the ration. The cattle were fed equal parts of alfalfa and a concentrate mixture. The fat was fed as part of the concentrate mixture replacing equal parts of ground milo by weight while the other additives were fed separately to each individual animal. No antioxidant was used. The alfalfa contained 18.5 mg. of carotene per pound. Additional feed analyses, management procedures, growth and slaughter data have been described previously (Bohman *et al.*, 1957).

Blood samples were taken initially and at approximately 40-day intervals during the experiment. These blood samples were analyzed for total cells, hemoglobin, phosphorus, vitamin A, carotene and fat. The following methods were used for the various chemical determinations: vitamin A and carotene as described by Kimble (1939); inorganic phosphorus by the method of Fiske and SubbaRow (1925) and blood fat by the method of Allen (1938). Total cell count was determined indirectly by diluting 0.075 ml. of whole blood to 25 ml. with normal saline and measuring the turbidity in a Coleman Jr. spectrophotometer at a wave length of 700 mμ. The sample was hemolyzed by the addition of a pinch of saponin and the

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hemoglobin determined colorimetrically as oxyhemoglobin at a wave length of 545 mμ.

At slaughter, the liver and seminal vesicles were freed from other tissues and weighed. In addition, the caudal lobe of the liver and the 9-10-11th rib cut were obtained. The rib cut was physically separated into its fat, lean and bone components. The separable fat, separable lean and liver sample were then ground and stored in moisture proof containers in the deep freeze until chemical analyses could be completed. The amount of dry matter, protein and fat were determined in the separable fat, separable lean and liver. In addition the ash, vitamin A, carotene, and total carbohydrates were determined on the liver sample. The samples were analyzed according to the following methods: The protein, fat, dry matter and ash were determined by AOAC methods (1955); the vitamin A and carotene were determined by the alkali digestion method described by Davis (1939); total carbohydrates were determined by difference.

All data were subjected to statistical analysis according to methods described by Snedecor (1956).

Results and Discussion

The feed additives investigated had no effect on the total cell count or the level of hemoglobin in the blood. From initial values the levels of hemoglobin and total cells decreased during the second and third bleedings, then returned to the initial values at the conclusion of the feeding period. The average values by periods are as follows expressed as millions of total cells per mm^3 and grams of hemoglobin per 100 ml. blood, respectively: initially, 8.58 and 16.6; 40 days on experiment, 7.90 and 15.5; 80 days, 7.38 and 15.9; 120 days, 8.06 and 16.9. Clegg and Cole (1954) found that the level of hemoglobin decreased when steers were implanted with stilbestrol, while the data of Erwin *et al.* (1956b) with oral stilbestrol confirms the data reported in this study.

The effect of dietary fat on some of the blood constituents is given in table 1. The level of plasma fat was influenced by the level of dietary fat and the length of time the animals were on experiment. The addition of animal fat in the diet increased the level of plasma fat but the response was not linear. Ten percent fat in the ration had no greater effect than five. As the experiment progressed, the level of plasma fat increased regardless of the level of dietary fat. Willey *et al.* (1952), and Bender and Maynard (1932), also found that dietary fat markedly increased the level of plasma fat.

After the initial bleeding, the level of plasma phosphorus increased. The increase was caused by a change of ration from grazed forage to feed lot rations with their higher level of phosphorus. Dietary animal fat also affected the level of plasma phosphorus. This effect was transitory when the animals were bled after 80 days on experiment, the level of plasma

TABLE 1. THE EFFECT OF DIETARY ANIMAL FAT ON BLOOD COMPOSITION

Level of fat in ration, %	0	5	10
Plasma fat, mg. %			
Initial	122	129	122
40 days	150	241**	222**
80 days	168	294**	256**
120 days	211	306**	306**
Plasma phosphorus, mg. %			
Initial	4.71	5.56	5.82
40 days	6.63	6.43	6.71
80 days	6.59	5.97*	5.94*
120 days	6.73	6.55	6.58
Plasma carotene, mcg./100 ml.			
Initial	530	542	544
40 days	288	323	233[a]
80 days	378	396	241**
120 days	453	483	234[a]
Plasma vitamin A, mcg./100 ml.			
Initial	56.8	58.9	59.5
40 days	50.0	47.7	42.2[a]
80 days	49.4	50.3	43.0*
120 days	55.9	59.0	49.6[a]

** Differs significantly from control, 0 level of fat, at P<.01.
* Differs significantly from control, 0 level of fat, at P<.05.
[a] Difference approaches significance at P .05.

phosphorus had decreased in the animals receiving supplementary fat but had returned to normal 40 days later. A similar transitory effect has been reported from this laboratory by Wade *et al.* (1956) when fat was fed to wintering beef cattle. At the same time (80 days) the plasma phosphorus was influenced by a significant stilbestrol x chlortetracycline interaction (table 2). The animals fed either chlortetracycline or stilbestrol had lower levels of plasma phosphorus than the controls. When both additives were fed together, the values approached those of the control animals. The biological significance of the effect of dietary treatments on plasma phosphorus is not readily apparent.

When fat was added to the ration, its effect on the level of plasma caro-

TABLE 2. THE EFFECT OF CHLORTETRACYCLINE AND STILBESTROL ON THE LEVEL OF PLASMA PHOSPHORUS AT 80 DAYS

Treatment	Chlortetracycline	None	Average
	Mg. per 100 ml. plasma		
Diethylstilbestrol	6.43[a]	5.91	6.17
None	5.52	6.79	6.15
Average	5.98	6.35	6.16

[a] Each value is the average value for 12 animals.

tene and vitamin A was inconsistent. Five percent added fat in the ration increased or maintained the level of both of these constituents at the same level as rations without supplementary fat. However, the inclusion of 10% fat in the ration significantly decreased both the plasma vitamin A and carotene (table 1) although it had no effect on feed consumption (Bohman *et al.*, 1957). Since no antioxidant was used, it is possible that some rancidity had developed which was responsible for the destruction of vitamin A and carotene. Rancidity was not detected organoleptically and all concentrate mixtures were fed within one week after mixing. Since the intake of carotene was relatively constant, rancidity would have its greatest

TABLE 3. THE EFFECT OF CHLORTETRACYCLINE AND STILBESTROL ON THE LEVEL OF PLASMA CAROTENE AND VITAMIN A

Feed additive	Chlortetracycline		Stilbestrol	
Level of additive	None	70 mg./day	None	10 mg./day
Plasma carotene, mcg. %				
Initial	516[b]	561	528	549
40 days	251	311*	300	262
80 days	298	379**	355	342
120 days	384	474[a]	413	445
Plasma vitamin A, mcg. %				
Initial	57.1	59.8	56.8	60.0
40 days	45.9	48.4	47.3	47.0
80 days	45.5	49.7	43.4	51.8**
120 days	52.4	57.3[a]	52.6	57.1[a]

* Difference is significant at P<.05.
** Difference is significant at P<.01.
[a] Difference approaches significance at P .05.
[b] Each value is the average of 24 animals.

effect upon the animals consuming the higher level of fat. When the level of dietary fat is increased, it usually results in a higher level of either plasma carotene or plasma vitamin A or both (Wade *et al.*, 1956; Russell *et al.*, 1942; Willey *et al.*, 1952). In this study the alfalfa (primary carotene source) and the concentrate mixture (source of added fat) were fed separately. These two ingredients were first mixed within the digestive tract of the animal. It suggests that carotene was probably destroyed in the rumen. Chlortetracycline had a "carotene sparing" effect as evidenced by the higher level of plasma carotene when the steers were fed this antibiotic (table 3). It is not known whether chlortetracycline affected the rumen microflora in preventing the destruction of carotene or the higher level of plasma carotene is the results of some other mechanism involving this antibiotic. The level of plasma vitamin A followed a similar pattern although the differences were not statistically significant. Stilbestrol increased the level of plasma vitamin A at the end of the second and third periods, but had no effect on the level of plasma carotene.

The initial level of both plasma carotene and vitamin A was high as the animals were changed from pasture to dry-lot rations at that time. As the level of dietary fat increased, the amount of vitamin A decreased in the liver. Liver carotene follows a similar pattern (table 4). The storage of vitamin A and carotene in the liver confirms the blood data and supports the hypothesis that fat caused a destruction of the vitamin or its precursor. The rancidity of the fat is the most likely mechanism. The animals

TABLE 4. THE EFFECT OF DIETARY ANIMAL FAT ON BODY TISSUES

Level of fat in ration, %	0	5	10
Composition of 9–10–11 rib cut, %			
Separable fat	27.82[a]	32.06	30.20
Separable lean	56.36	53.02	54.39
Bone	15.56	14.52	15.62
Composition of separable fat,			
Dry matter, %	87.75	88.60	88.23
Fat, % of dry matter	93.43	93.42	93.09
Protein, % of dry matter	5.85	5.73	5.83
Composition of separable lean,			
Dry matter, %	30.82	32.09	31.73
Fat, % of dry matter	28.02	30.45	29.47
Protein, % of dry matter	66.90	63.70	65.51
Liver weight, kg.	5.06	5.49*	5.37
Composition of liver,			
Dry matter, %	30.23	30.82	30.53
Fat, % of dry matter	20.11	20.52	21.21
Protein, % of dry matter	63.37	65.32	65.46
Ash, % of dry matter	1.83	2.71	2.39
Carbohydrates, % of dry matter	14.69	11.45	10.94
Carotene, mcg./gm. dry matter	25.7	20.6	18.6
Vitamin A, mcg./gm. dry matter	123.6	78.9**	61.4**

* Significantly different from control, 0 level of fat, at P 0.05.
** Significantly different from control, 0 level of fat, at P 0.01.
[a] Each value in the table is the average of 16 observations.

fed chlortetracycline and stilbestrol had higher levels of liver vitamin A although the differences were not significant (table 5). The effect of these additives on the levels of plasma carotene and vitamin A is thus supported. Erwin *et al.* (1956) found that dietary fat significantly increased carotene storage but had no effect on the storage of vitamin A. Their work does not mention if an antioxidant was used.

Neither chlortetracycline nor stilbestrol had any effect on the physical composition of the 9, 10 and 11th rib cut, the chemical composition of its component parts or the chemical composition of the liver (table 5). This agrees with the studies of Deans *et al.* (1956) and Kastelic *et al.* (1956) and in contrast to the work of Beeson *et al.* (1956) when oral estrogens were fed. Landagora *et al.* (1957) stated that oral feeding of

chlortetracycline appeared to favor fat deposition. This study fails to confirm his results.

In general, the addition of animal fat to the rations of fattening beef cattle increased the amount of fat in the carcasses of these animals (table 4). Because of the wide variation within groups on the same treatments, the differences were not statistically significant nor were

TABLE 5. THE EFFECT OF CHLORTETRACYCLINE ON BODY TISSUES

Feed additive	Chlortetracycline		Stilbestrol	
Level	None	70 mg./day	None	10 mg./day
Composition of 9–10–11 rib cut, %				
Separable fat	29.46[a]	30.59	30.13	29.92
Separable lean	54.78	54.42	54.15	55.03
Bone	15.75	14.72	15.42	15.05
Composition of separable fat,				
Dry matter	88.08	88.38	88.39	88.07
Fat, % of dry matter	92.78	93.84	93.74	92.88
Protein, % of dry matter	6.04	5.56	5.85	5.75
Composition of separable lean, %				
Dry matter	31.33	31.76	31.73	31.37
Fat, % of dry matter	29.35	29.28	29.43	29.20
Protein, % of dry matter	65.62	65.11	65.84	65.90
Liver weight, kg.	5.38	5.24	5.20	5.41
Composition of liver,				
Dry matter, %	30.34	30.72	30.42	30.63
Fat, % of dry matter	20.69	20.54	21.22	20.01
Protein, % of dry matter	65.39	64.04	63.66	65.77
Ash, % of dry matter	2.30	2.16	2.30	2.16
Carbohydrates, % of dry matter	12.08	12.45	12.26	12.45
Carotene, mcg./gm. dry matter	20.5	22.8	21.9	21.4
Vitamin A, mcg./gm. dry matter	80.5	95.4	83.5	92.4

[a] Each value in the table is the average of 24 observations.

carcass grades influenced (Bohman *et al.*, 1957). On the average in this experiment, cattle receiving animal fat had 2 to 4% more separable fat and 1 to 3% less lean in the 9–10–11th rib cut; 1.5 to 2.5% more fat and 1.5 to 3.0% less protein in the separable lean. Willey *et al.* (1952) reported that a high fat intake decreased the deposition of fat in the 9–10–11th rib cut. The liver was significantly heavier for the cattle receiving 5% fat in their rations. This was not reflected by any difference in the composition of the liver (table 4). Since the cattle that were fed fat were heavier, the increased weight of this organ probably reflects only the difference in body weight at slaughter.

Stilbestrol significantly increased the weight of the seminal vesicles. This has been reported earlier (Clegg and Carroll, 1957). However, fat

TABLE 6. THE EFFECT OF STILBESTROL AND ANIMAL FAT ON THE WEIGHT OF THE SEMINAL VESICLES

Fat levels	0	5	10	Av.
		Gm.		
Stilbestrol	31.2[a]	27.2	25.4	27.9
None	8.5	10.9	10.3	9.9
Difference	22.7[b]	16.3	15.1	18.0**

[a] Each figure represents the average of 8 observations.
[b] Stilbestrol × fat interaction significant at $P<.05$.
** Difference is significant at $P<.01$.

decreased this effect (table 6). As the level of fat increased in the ration, the difference between the weight of the seminal vesicles from animals receiving stilbestrol and their controls decreased. This interaction was statistically significant. This confirms previous work at this station (Bohman *et al.*, 1957). In an earlier paper, it was suggested that stilbestrol may have been dissolved in dietary fat and excreted as part of the undigested lipids. These data support that hypothesis.

Since the level of blood fat increased during the fattening period and in view of the dynamic state of body fat, it appeared that blood fat might be related to the deposition of body fat and reflect the amount deposited in the animals body. Since blood fat was influenced by dietary treatments, correlations of blood fat with certain components of body composition and production factors were computed on a within-group basis. These data are shown in table 7. The correlations of final plasma fat with the percent fat in the 9–10–11th rib, the separable lean, or the liver did not differ significantly from zero. However, initial blood fat was significantly correlated with rate and economy of gain. These values are approximately of the same degree of magnitude of those reported earlier by Kidwell *et al.* (1957) for glutathione but in the reverse order. Although these values and those reported by Kidwell *et al.* are significant, they

TABLE 7. CORRELATIONS OF PLASMA FAT AND OTHER CARCASS AND PRODUCTIVE DATA

Item	Plasma fat	
	Initial	Final
Fat, 9–10–11 rib, %	.....	0.281
Liver fat, %	.....	0.021
Fat in lean, %	.....	0.274
Initial plasma fat, %	.....	0.569**
Av. daily gain	0.430**	0.356**
Hay per lb. of gain	−0.40**	−0.285
Grain per lb. of gain	−0.391*	−0.251

*, ** Significant at P .05 and P .01, respectively.

are not high enough to serve as a tool in selecting cattle that will make rapid and efficient gains in the feedlot. It does suggest that some physiological factor may exist that will be more related to growth.

Summary

Forty-eight Hereford steers were assigned treatments for a 120-day feeding period in a 2x2x3 factorial designed experiment to determine the main effects and interactions of feeding two levels of chlortetracycline, two levels of stilbestrol and three levels of animal fat upon the composition of the blood, carcass and liver.

Dietary animal fat significantly increased the level of plasma fat and decreased the level of plasma carotene, plasma vitamin A, liver carotene and liver vitamin A. It also increased the percent of fat in the carcass but not significantly. Dietary animal fat significantly decreased the effect of stilbestrol on the weight of the seminal vesicles. Animal fat, chlortetracycline and stilbestrol temporarily decreased the level of plasma phosphorus.

Chlortetracycline increased the level of plasma carotene and stilbestrol increased the level of plasma vitamin A. Stilbestrol also significantly increased the weight of the seminal vesicles.

Neither chlortetracycline or stilbestrol had any effect on the composition of the carcasses or livers of the experimental cattle.

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THE UTILIZATION OF COTTON GIN TRASH BY GROWING AND FATTENING STEERS [1]

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IN the ginning of cotton, 50 to 75% of the harvested material is trash, such as leaves, stems, lint and a few seeds. This material presents a fire hazard and consequently incinerators have been constructed at many cotton gins to relieve the surplus of cotton gin trash from the ginning process. The potential importance of this material as a roughage for livestock can only be appreciated when one considers that there are over one million tons of gin trash annually available in the cotton producing areas of the United States. At the present time, little of this material is used as livestock feed.

In the past few years much interest has been shown in the utilization of low-quality roughages such as corn cobs (Beeson *et al.*, 1952), wheat straw (Erwin *et al.*, 1955), and prairie hay (Gossett and Riggs, 1956). Dehydrated alfalfa (Bentley *et al.*, 1952) as well as an appropriate mineral balance has been used in addition to a protein source for providing better utilization of low-quality roughages.

Results of earlier studies with stilbestrol implantation (Clegg and Cole, 1954) showed that increased steer gains were obtained at the expense of carcass quality. More recently, lower stilbestrol levels (O'Mary *et al.*, 1956) have shown promise as a growth stimulator with little deleterious carcass effects. However, few reports exist in which stilbestrol has been reimplanted at low levels for prolonged periods. Furthermore, limited amount of work has been reported where steers that consumed various quantities of high and low quality roughages were implanted with stilbestrol.

The possibility of gin trash serving as a roughage source for growing and fattening steers was considered in two studies along with stilbestrol implants.

Procedure

Experiment A. Since the palatability and feeding value of unprocessed cotton gin trash was initially unknown, 30 steers, individually penned, were divided into five treatment groups and treated as follows during an 83-day growing period: (1) hegari silage, (2) silage-milo grain-gin trash,

[1] Experiment A was initiated by R. M. Richard.

[2] Stilbestrol pellets used in this experiment were furnished by Chas. Pfizer and Co., through the courtesy of W. C. Sherman.

[3] The authors acknowledge the statistical assistance of H. Tucker, Experiment Station statistician.

[4] The assistance of W. Hall, Herdsman, for feeding the experimental animals is acknowledged. Arizona Agricultural Experiment Station Paper No. 429.

(3) milo grain-gin trash, (4) gin trash, and (5) gin trash-molasses. In addition, all animals were fed 4 lb. daily of supplement A shown in table 1. During the following 76-day fattening period, milo grain (11 lb. per head daily) was added to the rations of groups (4) and (5). The rest of the respective treatments remained unchanged.

Average daily gains, feed consumption, feed efficiency, transit shrink (130 miles to market), dressing percentage and federal carcass grades were subjected to the multiple range test for significance (Duncan, 1955).

Experiment B. One-hundred and eight yearling steers were randomly allotted to six pens of 18 animals each. Six ratios of hegari silage:cotton gin trash (100:0, 80:20, 60:40, 40:60, 20:80, and 0:100) were fed during

TABLE 1. CONSTITUENTS OF SUPPLEMENTS, PERCENT

Ingredients	Supplemental designations		
	A	B[a]	C[a]
Cottonseed meal	46.9	41.7	12.0
Dehydrated alfalfa	46.9	41.7	12.0
Molasses	....	10.0	10.0
Barley	....	....	32.5
Milo	....	....	32.5
Bone meal	4.8	4.1	1.0
Salt (trace mineral)	1.4	2.5	0.5
Vitamin A, I.U. per lb.	2700 I.U.	4000 I.U.	1100 I.U.

[a] Compounded into one-half inch pellets.

a 91-day growing period and a fattening period. Shown in table 1 are the constituents of supplements B and C fed at the rate of 4 and 12 lb. per steer daily during the growing and fattening periods, respectively.

Each pen of steers was randomly selected for three sub-treatments of stilbestrol implantation. Six steers in each pen served as controls while the remaining two groups of six steers were implanted with the following amounts of stilbestrol: (1) 36 mg. at 0 and 91 days, (2) 12 mg. at 0, 30, 60, 91, and 120 days.

Two of the heaviest steers in each sub-treatment group were marketed at 63 days of the fattening period. The remaining steers were fed 47 days longer. All data including the average daily gain, dressing percentages and carcass grades were treated for statistical significance by analysis of variance and the multiple range test of Duncan (1955). No division for statistical treatment was made for animals marketed at different times.

Results and Discussion

Experiment A. Tables 2 and 4 show the results and statistical treatment of the data from Experiment A. During the growing period the rates of gain in the steers fed silage (Group 1) were not significantly different

from those produced by the animals fed silage-grain-gin trash (Group 2) or grain-gin trash (Group 3). However, the above rations resulted in faster gains than Groups 4 and 5. Unprocessed gin trash proved palatable, for the all-silage fed steers (Group 1) consumed significantly less dry matter daily than those animals in any other group. No significant difference was found in feed efficiency between the silage fed group and Groups 2 and 3. However, silage produced more efficient gains than gin trash (Group 4) or gin trash-molasses (Group 5).

TABLE 2. RATES OF GAIN, FEED CONSUMPTION, AND FEED EFFICIENCY AS INFLUENCED BY TYPE OF RATION IN EXPERIMENT A

	Experimental rations									
	83-Day growing period (A)					76-Day fattening period (B)				
Groups number	1	2	3	4	5	1	2	3	4	5
Feed per day per steer, lb.										
Silage	38.0	24.2	....	....	....	46.1	26.7	....	....	....
Gin trash	....	3.9	9.6	....	....	....	4.4	10.0	11.1	11.1
Grain	....	3.9	9.6	12.2	12.7	....	4.4	10.0	11.1	11.1
Supplement	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Molasses	....	....	....	....	1.5	....	....	....	....	1.5
Av. dry matter/day, lb.	13.3	16.4	21.6	15.9	17.5	15.2	18.5	22.3	19.9	21.4
Number steers	6	6	6	6	6	6	6	6	6	6
Av. initial wt., lb.	558	570	587	561	552	735	757	763	652	654
Av. final wt., lb.	735	757	763	652	654	858	916	914	828	827
Av. daily gain, lb.	2.14	2.25	2.13	1.09	1.23	1.62	2.09	1.99	2.32	2.28
Dry matter/100 lb. gain, lb.[a]	624	728	1029	1591	1496	960	906	1164	873	953
Wt. lost in marketing (incl. transit loss), %						47.6	43.7	40.5	44.0	44.3
Shrink (24 hours incl. transit loss), %						6.2	6.7	3.2	4.5	6.7
Dressing % (based on off-truck wt. and warm carcass wt.						55.7	60.4	61.5	58.7	59.7
Carcass grade[b]						1.83	1.67	1.30	2.0	1.83

[a] Dry matter percent: hegari silage, 25; cotton gin trash, 93; milo grain, 93; supplement, 93; molasses, 70.
[b] Choice=1.0, Good=2.0.

During the fattening period no significant difference in rates of gain were found between Groups 4, 5 and 2. However, the steers fed 50% grain-50% gin trash (Group 3) and silage (Group 1) gained at a slower rate. Groups 3 and 5 consumed more dry matter daily than the animals in the other groups and the silage fed groups consumed significantly less. Apparently the molasses addition to gin trash during the growing and fattening periods proved to be of no value in increasing rates of gain of feed utilization. These results are similar to those reported by Klosterman *et al.*, (1956) who added molasses to poor quality hay rations.

All animals were shrunk for 24 hours and then trucked 130 miles to market. Steers in Group 3 shrunk in transit significantly less than the other treated groups of animals. The average shrink of the molasses-fed

steers was as high as any group; that was due in part to a probable weighing error for one steer was "well out of line". Silage-fed steers resulted in larger transit shrink than grain-gin trash fed animals.

No significant difference in dressing percentage was found between Groups 2 and 3. However, a lower dressing percentage occurred in the other groups. Silage-fed steers dressed significantly less than the other groups.

Experiment B. Tables 3 and 5 show the results and statistical treatment of the data from Experiment B. During the growing period no significant difference was found between the rates of gain in steers fed all silage and silage:gin trash ratios of 80:20 and 60:40 during the growing period (table 3). However, when gin trash was increased in ratios of 40:60, 20:80, and 0:100 significantly slower gains were found as compared to silage alone. Furthermore, rations of all-gin trash roughage resulted in significantly slower rates of gain than any other ration that contained silage. During the growing period, the implantation of 36 mg. initially or 12 mg. of stilbestrol monthly significantly increased rates of gain 45 and 38%, respectively. Neither the quality of roughage nor the method of hormone administration altered the response of the steers to stilbestrol.

Twelve pounds of supplement B were fed per steer daily during the fattening period. However, the silage:gin trash ratios remained unchanged. The rates of gains of steers fed silage:gin trash ratios of 80:20, 60:40, 40:60 during the fattening period were not significantly different from those fed all-silage. Table 3 shows that inferior gains were obtained in steers fed all-gin trash and in animals fed 20% silage-80% gin trash.

During the fattening period the two methods of stilbestrol implantation resulted in 17.4 and 15.7% increased gains. These differences were statistically significant from the untreated animals, but not different from each other. Furthermore, no ration x stilbestrol interaction was found. However, side effects (high tail heads) were observed after 30 to 40 days of the fattening period. Possibly there was an insufficient amount of time between implantations.

Although the animals in this study were marketed similarly to those in experiment A, the transit shrink shown in table 3 was inconsistent with treatment. Upon slaughter, no significant differences in dressing percentage could be attributed to treatment. Regarding carcass grades, the all-silage fed steers graded significantly higher than any other group. The all-gin trash fed steer carcasses were not statistically different from those treated with 20% silage: 80% gin trash, but significantly lower grade than those in the other groups. The implantation of stilbestrol also reduced carcass grades significantly. This difference was probably due in part to the improper implantation of the hormone. The implantation of 12 mg. monthly depressed carcass grades the most, while the reimplantation of 36 mg. was intermediate. No stilbestrol x quality of roughage interaction was found.

TABLE 3. EFFECT OF ROUGHAGE RATIOS AND STILBESTROL IMPLANTATION DURING THE GROWING AND FATTENING PERIOD: EXPERIMENT B

	Growing period						Fattening period					
Silage: gin trash ratio	100:0	80:20	60:40	40:60	20:80	0:100	100:0	80:20	60:40	40:60	20:80	0:100
No. steer days	1638	1638	1638	1638	1246	1638	1734	1734	1734	1734	1624	1734
Av. daily gain; lb.												
No DES	1.65	1.34	1.24	1.20	1.24	0.65	2.06	1.89	1.97	1.67	1.67	1.77
12 mg./mo. DES	1.91	2.02	1.77	1.67	1.77	0.92	2.04	2.17	2.45	2.15	1.88	2.06
36 mg. DES	2.06	1.83	2.09	1.75	1.69	1.19	2.23	2.14	2.24	2.01	1.75	1.88
Feed/steer/day, lb.												
Silage	43.6	32.7	24.5	16.1	8.2	....	32.3	26.7	20.8	12.6	7.2	....
Gin trash	....	2.8	5.4	8.1	11.0	14.5	....	2.1	4.6	6.3	9.7	11.7
Pellets	4.0	4.0	4.0	4.0	4.0	4.0	11.9	11.9	11.8	11.8	12.0	11.8
Av. D.M./day/steer, lb.	18.4	17.7	17.6	17.5	17.7	18.5	22.7	22.9	23.3	22.3	24.1	23.5
D.M./100 lb. gain, lb.	9.8	10.1	10.4	11.4	11.3	20.1	10.2	10.7	10.4	11.1	13.8	12.5
Transit shrink, %												
No DES							2.7	3.0	1.3	3.5	3.6	3.5
12 mg./mo. DES							2.2	2.5	2.8	2.7	2.8	0.5
36 mg. DES							1.9	2.9	3.7	3.0	3.2	2.8
Dressing, %												
No DES							61.8	61.0	59.7	60.0	58.9	57.9
12 mg./mo. DES							61.3	60.0	59.9	60.3	59.1	58.2
36 mg. DES							61.9	62.1	60.5	60.7	60.9	59.6
Carcass grade[a]												
No DES							1.83	2.67	3.50	3.67	3.67	5.67
12 mg./mo. DES							3.16	3.67	4.83	4.00	4.67	5.50
36 mg. DES							2.83	3.17	4.33	4.50	4.83	4.50

[a] Low choice=1; High good=2; Medium good=3; Low good=4; High standard=5; Medium standard=6; Low standard=7.

TABLE 4. ANALYSES OF VARIANCE OF DATA FROM EXPERIMENT A

Source	d/f	Av. daily gain		D.M./steer/day		D.M./100 lb. gain		Loss in transit, %	Dressing %
		G	F	G	F	G	F		
Treatments	4	1.853**	.505**	53.71**	44.3**	1150241**	77194*	31.23**	14.94*
Error	25	.081	.094	1.36	1.42	140246	28267	1.88	4.52

G=Growing period; F=Fattening period.
* P .05.
** P .01.

Breidenstein *et al.* (1956) reported that stilbestrol reimplantations of 20 mg. monthly in heifers did not influence either live slaughter grade or carcass grades. However, 12 mg. of stilbestrol implanted monthly produced lower slaughter grades and significantly lower carcass grades (table 3) in this study. The differences in stilbestrol response between these studies merit consideration.

Summary

Initially, it was found with individually fed steers that unprocessed gin trash was as palatable as silage. The addition of molasses did not add to palatalibity, rates of gain, or feed efficiency. Because the silage-gin trash-grain mixture produced desirable gains, a second study was initiated that involved six ratios of silage:gin trash fed to groups of steers during a growing and a fattening period. It was found that gin trash, when fed as part of the roughage, was equal to silage in the production of steer gains. Stilbestrol resulted in markedly increased gains although no difference was observed between animals implanted with 36 mg. at 0 and 91 days and 12 mg. of stilbestrol monthly. A reduced carcass grade was found due to stilbestrol as well as to progressive increases of dietary gin trash. The stilbestrol and ration effects on rates of gain and carcass grades were independent of each other.

TABLE 5. ANALYSIS OF VARIANCE OF DATA FROM EXPERIMENT B

Source	d/f	Av. total gain growing	Av. daily gain fattening	Dressing %[b]	Carcass grade[b]
DES	2	22586**	1.125**	14.46	7.9**
Diet	5	17071**	.709**	20.97	16.5**
Diet × DES	10	860	.178	2.41	1.5
Error[a]	89	800	.126	13.88	1.6

[a] A group mean was used in place of one animal that was removed from the study; therefore, the error mean square was calculated with one less degree of freedom.
[b] Error mean square of dressing percentage and carcass grade was calculated with 85 degrees of freedom because four steers were not slaughtered.
* P .05.
** P .01.

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STILBESTROL RESPONSES IN LAMBS FED RATIONS DIFFERING IN CALORIE TO PROTEIN RATIOS [1, 2]

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STILBESTROL (diethylstilbestrol) administered either orally or subcutaneously (Hale *et al.*, 1955; Light *et al.*, 1956; Wilkinson *et al.*, 1955) has been shown to increase liveweight gains in fattening lambs, to increase feed efficiency per unit of gain, and to have either no observable influence or a depressing influence upon carcass value. In general, response to stilbestrol reflected in carcass measurements has been more variable in experiments with lambs than in those with beef cattle. A preliminary analysis of feedlot and carcass data available in the literature suggested that a part of this variability might be due to differences in the protein and energy make-up of the rations. Two preliminary experiments with lambs (Preston, 1957) further suggested interrelationships between ration energy and protein with respect to response from stilbestrol. The purpose of this experiment was critically to evaluate the response of lambs to different amounts of stilbestrol in rations differing in amounts of protein and energy, and differing in ratios of energy to protein.

Experimental Procedure

The general procedure consisted of individually feeding 144 lambs 18 different rations and noting liveweight gains, feed consumption and any unusual performance. Following the feeding period, the lambs were slaughtered and physical and chemical measurements were made on each carcass and on selected tissues and glands.

The 18 rations consisted of three levels of stilbestrol (0, 0.3 and 0.6 mg. per lb. of ration), three levels of crude protein (9, 13 and 17% of the total ration) and two energy levels (530 and 665 Calories of estimated net energy, ENE, per pound, of ration). A 3x3x2 factorial design was used in this experiment based upon the above three variables for purposes of gaining information on each and also possible interrelationships between the different variables. Table 1 lists the six rations containing no stilbestrol. The other 12 rations were similar except for the respective stilbestrol additions. Individual feeding was carried out in crates where the lambs

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[2] A portion of the thesis presented to the Graduate School at Iowa State College by the senior author in partial fulfillment of the requirements for the degree Doctor of Philosophy, June 1957.

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were confined for two 2-hour periods daily. The remainder of the day the lambs were allowed to run together in a small paddock where water and salt were available at all times.

Sufficient feed to insure a uniform supply was set aside at the beginning of the experiment. The feeds were analyzed for protein content, and their net energy values were estimated from tabular material presented by Morrison (1956). All rations were mixed mechanically and fed as single mixtures.

TABLE 1. LAMB FATTENING RATIONS CONTAINING NO STILBESTROL

Energy level	Feed ingredients	Percent crude protein in total ration[a]		
		9.3	13.2	17.2
		%	%	%
530 Cal. ENE per lb. ration	Ground corn cobs	40.0	40.0	40.0
	Ground alfalfa hay	20.0	20.0	20.0
	Blackstrap molasses	10.0	10.0	10.0
	Cracked corn	23.4	13.0	2.4
	Soybean meal	5.9	16.5	27.2
	Dicalcium phosphate[b]	0.2	0.1	....
	Monosodium phosphate[b]	0.5	0.4	0.4
	Cobalt sulfate[c]			
665 Cal. ENE per lb. ration	Ground corn cobs	10.0	10.0	10.0
	Ground alfalfa hay	20.0	20.0	20.0
	Blackstrap molasses	10.0	10.0	10.0
	Cracked corn	59.0	49.1	37.7
	Soybean meal	0.5	10.5	22.2
	Dicalcium phosphate[b]	0.2	0.1	....
	Monosodium phosphate[b]	0.3	0.2	0.1
	Cobalt sulfate[c]			

[a] N×6.25 and expressed on an air-dry basis.
[b] Dicalcium and monosodium phopshates were added in order to standardize the Ca and P contents of all rations at a calculated level of .40 and .30% respectively.
[c] Cobalt was supplemented at the rate of 1 ppm in all rations.

Two groups of 72 Western wether lambs were selected for this experiment. One group was fed during the summer of 1956 and the other was fed during the fall and early winter of 1956–57. The two groups of lambs were assigned to the various rations by outcome groups based upon initial fatness or finish and weight. Finish at this time was appraised by a committee of three working independently such that the lambs could be divided into two groups. Each finish group was then divided on a weight basis to give four outcome groups from which the lambs were randomly assigned to the 18 ration treatments making a total of eight replications on each of the treatments.

The length of time the lambs remained on experiment varied but was determined on the following basis. An average final body weight of 108 lb.

was selected. The difference between the average initial weight of the 18 lambs on a given replication and 108 lb. was the weight which every lamb within this replication was required to gain before being slaughtered. Thus, if a lamb which weighed 74 lb. initially was included in a replication which averaged 78 lb., this lamb as well as all the other 17 lambs in this group was required to gain 30 lb. The average initial weight of all lambs was 81 lb.

Lambs reaching their predetermined weight gain were graded on foot and slaughtered at the campus meats laboratory. Dressing percentages as well as carcass grades were determined in the conventional manner on the 48-hour chilled carcasses. The carcasses were then separated into standard wholesale cuts and their weights were expressed as percentages of the total carcass weight. The rack was then separated physically into lean, bone and fat. Following the weighing of these three separations, the longissimus dorsi muscle was dissected free of external fat, ground, and analyzed for moisture and fat. Various organs and glands were weighed at the time of slaughter. These included the seminal vesicle, bulbo-urethral, adrenal, anterior pituitary, thyroid, kidney, and the liver. Blood samples were collected just prior to slaughter from half of the lambs on each ration treatment. They were analyzed for serum glucose and serum amino acid nitrogen.

Results and Discussion

Feed Lot Results. Stilbestrol increased liveweight gains in the lambs, table 2, on each of the six different rations with one exception, namely, the high protein (17%)—low energy (530 Calories/lb.) ration. The average daily liveweight gain for the six lots of lambs receiving no stilbestrol was 0.35 lb. as compared to 0.43 lb. for those receiving both the 0.3 and 0.6 mg. levels of stilbestrol. This represented a 23% average stimulation in liveweight gain which was statistically significant ($P<0.01$). Lambs receiving the 13 and 17% protein rations made greater average liveweight gains (0.42 lb. each) than those receiving the 9% ration (0.37 lb.). Similarly lambs receiving the high energy ration made a higher average liveweight gain (0.44 lb.) than those receiving the low energy ration (0.36 lb.). These differences due to the protein and energy levels proved statistically significant at the 1% level.

Results in table 2 suggest interacting influences upon liveweight gains between protein and energy levels, and between stilbestrol and energy levels. The interaction between protein and energy levels was such that increasing the protein level in the 530 Cal. ration resulted in no increase in average liveweight gain, whereas increasing the protein level from 9% to 13 and 17% in the 665 Cal. ration increased gains by 25 and 44%, respectively. This suggested that on the low energy ration 9% protein supported maximum gains, while on the high energy rations 13% or more of protein was needed. This interaction was statistically significant at the 1% level. The interaction between stilbestrol and energy levels suggested

an interesting trend even though its statistical significance was of a low order. The average daily increase in liveweight gain produced by stilbestrol on the low and high energy rations was 0.05 and 0.10 lb., respectively. Thus a greater gain stimulation on high energy rations is indicated.

There was no interaction between the levels of protein and stilbestrol. It is interesting, however, to note the type of response to stilbestrol feeding

TABLE 2. FEED LOT RESULTS WITH LAMBS FED VARYING LEVELS OF ENERGY, PROTEIN, AND STILBESTROL

Energy level	Protein level	Stilbestrol level	Av. daily gain	Av. daily feed intake	Feed per lb. of gain[a]	Live grade[b]
Cal.	%	mg.	lb.	lb.	lb.	
		0	.32	3.6	11.5	5.8
	9	0.3	.43	3.7	8.9	6.0
		0.6	.37	3.6	10.6	4.5
		0	.34	3.7	10.9	5.6
530	13	0.3	.41	3.7	9.4	6.0
		0.6	.41	3.8	10.2	5.2
		0	.32	3.9	12.8	6.4
	17	0.3	.30	3.4	12.3	5.1
		0.6	.34	3.8	12.0	5.6
		0	.30	3.0	10.3	6.1
	9	0.3	.38	3.2	8.6	5.9
		0.6	.39	3.3	8.6	6.4
		0	.41	3.3	8.9	6.4
665	13	0.3	.46	3.3	7.6	7.2
		0.6	.49	3.5	7.3	6.9
		0	.43	3.5	8.2	7.0
	17	0.3	.57	3.9	6.8	7.2
		0.6	.57	3.6	6.4	6.5

[a] Discrepancies between the values shown in this column and those which can be calculated using the average feed consumption and daily gain figures are due to the fact that those in this column are averages of the individual data.

[b] Choice plus, 9; choice, 8; choice minus, 7 etc.

at the different protein levels within each energy level. On the low energy ration, stilbestrol gave an increased gain on the 9% protein ration, slightly smaller increases on the 13% ration, and no increase on the 17% ration. On the high energy ration, the greatest response to stilbestrol was obtained on the 17% protein ration.

Daily feed consumption by the lambs was affected by ration protein and energy levels ($P<0.01$), and by their interaction ($P=0.04$), but was not affected by stilbestrol. The average daily feed intake on the low and high energy rations was 3.7 and 3.4 lb., respectively; it increased from 3.4 to 3.5 and 3.7 lb. per day on the 9, 13 and 17% protein rations, respectively.

This latter increase was brought about primarily by the increase in feed consumption on the high energy rations (3.2, 3.4, 3.7 lb., respectively), which accounts for the significant energy-protein interaction noted in feed consumption.

Feed efficiency was favorably influenced by both stilbestrol and energy level. Stilbestrol reduced the amount of feed required per lb. of gain from 10.4 lb. for the lambs which received no stilbestrol to 8.9 and 9.2 lb., respectively, for those receiving the 0.3 and 0.6 mg. amounts. This response was fairly uniform over all levels of energy and protein. As would be expected, less feed per lb. of gain was required with the high energy ration than with the low energy ration. The effect of level of protein upon feed efficiency was variable. When the protein content of the rations was increased from 9 to 13%, feed required per lb. of gain was decreased from 9.7 to 9.0 lb.; however, when the protein content was raised to 17% it was increased again to 9.8 lb. This increase was due solely to the increase observed on the low energy ration, since on the high energy ration there was a further decrease. This opposite effect at the two levels of energy accounts for the significant protein-energy interaction ($P<0.01$).

The live grades of the lambs (table 2) appeared to be influenced most by the energy level of the ration. This treatment effect proved significant statistically ($P<0.01$) and amounted to about one-third of a grade. Increasing amounts of protein in the ration had a slight beneficial influence ($P=0.07$). The lambs fed the 17% protein ration graded about one-sixth of a grade higher than the lambs fed the 9% ration. Stilbestrol influenced live grade little, if at all ($P=0.22$).

Carcass Results. Dressing percentage of the slaughtered lambs was influenced most by the energy content of the rations (table 3). Lambs receiving the high and low energy rations had average dressing percents of 52.9 and 49.7, respectively. This difference was statistically significant ($P<0.01$), and much of it could be accounted for by the greater weight of the digestive tract contents of the lambs fed the low energy rations. The overall effect of stilbestrol was a slight lowering ($P<0.01$) of dressing percentage from 52.0 to 51.0%. Ration protein on the other hand had a slight beneficial influence on dressing percentage ($P=0.03$) increasing it from 50.7 in the 9% ration to 51.9 and 51.4 in the 13 and 17% rations, respectively. Cooler shrink was not affected by the treatments.

Carcass grades were influenced somewhat by the energy content of the ration ($P<0.01$); however, this difference amounted to only one-sixth of a grade in favor of the high over the low energy treatment. Stilbestrol tended to decrease carcass grade slightly on the low energy ration while having no effect in the high energy rations; however, all differences were too small to be statistically significant.

The wholesale cut percentages in general were not affected by the feeding of stilbestrol. The only wholesale cut possibly affected by the feeding of stilbestrol was the rack. The slight decrease in the percentage of rack to

total carcass appeared to be accounted for by an increase in the percent of leg and shoulder; however, the differences in these wholesale cuts were not statistically significant. It would appear, therefore, that stilbestrol did not alter the distribution of the weight in these carcasses. Protein levels had no effect upon the percentage of the wholesale cuts. Energy level, however, did appear to effect slightly the amount of leg and rack. On the higher energy intake, there appeared to be a little more rack and less leg. This is probably a result of increased rate of fat deposition in the rack

TABLE 3. CARCASS EVALUATIONS OF LAMBS FED VARYING LEVELS OF ENERGY, PROTEIN, AND STILBESTROL

Energy level	Protein level	Stilbestrol level	Av. dressing percent	Av. cooler shrink	Av. carcass grade[a]
Cal.	%	mg.		%	
		0	50.4	2.8	6.9
	9	0.3	48.4	2.8	6.1
		0.6	48.3	3.1	5.8
		0	50.8	2.6	6.8
530	13	0.3	49.4	3.0	6.9
		0.6	50.8	3.1	6.2
		0	50.2	3.6	6.6
	17	0.3	49.1	2.3	6.4
		0.6	50.2	2.8	6.6
		0	52.8	2.7	6.8
	9	0.3	52.0	2.8	7.0
		0.6	52.5	3.0	7.2
		0	54.4	2.3	7.2
665	13	0.3	53.3	2.7	7.1
		0.6	52.6	2.8	7.2
		0	53.4	2.3	7.6
	17	0.3	52.4	2.7	6.8
		0.6	53.0	2.8	7.1

[a] See footnote b, table 2.

compared to that of the leg as the animal increases in fatness (Hankins and Titus, 1939).

Composition of the Rack. Table 4 shows the effect of various treatments upon the physical and chemical composition of the rack. The racks from the lambs receiving the high energy ration carried significantly ($P<0.01$) more fat (27.2%) compared to those receiving the low energy ration (23.9%). They also had less lean (51.7% as compared to 53.2%). Neither stilbestrol nor the protein level significantly influenced the physical composition of the rack when the average overall effects were analyzed statistically. There did appear, however, to be an interaction between stilbestrol and protein on the high energy ration. With the 9% protein-high

energy ration, increasing stilbestrol increased the amount of separable fat (25.6, 27.5 and 29.4%). With the 13% protein-high energy ration, increasing levels had essentially no influence upon separable fat (28.1, 27.6 and 27.6%) whereas, on the 17% protein-high energy ration, increasing levels had a reversing influence on the amount of separable fat (28.6, 24.9 and 25.5%). The same general picture appeared for the low energy

TABLE 4. INFLUENCE UPON THE PHYSICAL AND CHEMICAL COMPOSITION OF THE RACKS FROM LAMBS FED VARYING LEVELS OF ENERGY, PROTEIN AND STILBESTROL

Energy level	Protein level	Stilbestrol level	Physical comp. of rack		Chemical comp. of long dorsi M.	
			lean	fat	moisture	fat[a]
Cal.	%	mg.	%	%	%	%
		0	53.5	24.5	73.8	18.4
	9	0.3	53.1	23.4	74.4	17.9
		0.6	52.9	23.0	74.5	17.1
		0	52.8	25.4	73.4	19.9
530	13	0.3	53.6	23.4	74.3	16.4
		0.6	53.5	24.4	74.3	16.4
		0	51.9	25.9	74.2	16.4
	17	0.3	53.4	24.0	74.2	17.0
		0.6	53.9	21.6	74.1	17.6
		0	52.8	25.6	73.9	17.6
	9	0.3	50.4	27.5	73.4	19.9
		0.6	50.4	29.4	73.2	19.9
		0	51.9	28.1	73.7	19.1
665	13	0.3	51.6	27.6	74.2	17.0
		0.6	51.8	27.6	74.4	14.1
		0	50.6	28.6	74.3	17.9
	17	0.3	52.8	24.9	74.7	16.4
		0.6	52.9	25.5	74.3	16.4

[a] Values are expressed on a dry matter basis.

rations; however, with increasing level of stilbestrol in the 9% protein ration there was a slight decrease in the amount of separable fat (24.5, 23.4 and 23.0%). The addition of stilbestrol decreased the separable fat more on the 17% protein-low energy ration than on any other combination of protein and energy (25.9, 24.0 and 21.6%).

The overall influence of the various treatments upon the chemical composition of the longissimus dorsi M. was probably not significant ($P>0.25$). However, the amount of moisture and ether extract did appear to be slightly influenced by the levels of protein ($P=0.23$ and 0.13, respectively). For the 9, 13 and 17% protein rations, the percentage figures for moisture and ether extract were 73.9, 74.1 and 74.3, and 18.5,

17.1 and 16.9, respectively. When these moisture figures were converted to a fat-free basis, they became almost identical, namely, 77.6, 77.5 and 77.6%, respectively. Likewise, when the moisture figures for the three levels of stilbestrol (0, 0.3 and 0.6 mg.), were expressed on a fat-free basis, they became 77.6, 77.7 and 77.5%, respectively. From these results it would appear that any change in the moisture content of this muscle was strictly a function of its fat content. One might expect an increase in the fat content of the longissimus dorsi M. on the higher energy ration when

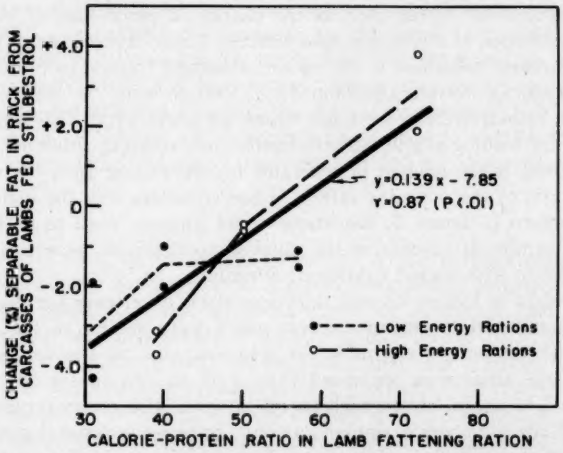

Figure 1. Stilbestrol influences upon carcass fatness in lambs fed rations differing in Calorie to protein ratios. (The Calorie: protein ratio is defined as the estimated net energy (ENE) in Calories per lb. of ration divided by the percentage of protein in the ration).

carcasses showed a higher content of separable fat; however, this was not the case. The average fat content of this muscle from lambs on the low and high energy rations was 17.5 and 17.6%, respectively. The reason is not apparent, since the racks from the high-energy lambs contained 12.5% more separable fat.

The most revealing influence upon carcass composition was the apparent reversible influence of stilbestrol in bringing about either a fatter or a leaner lamb carcass depending upon whether the ration had a relatively wide or narrow energy to protein ratio. This is illustrated in figure 1 in which the Calorie to protein ratio (ENE in Calories per pound of ration divided by the percentage of protein in the ration) was plotted against the change in percent of separable fat in the racks of lambs receiving stilbestrol. Statistical analysis of these data from 6 control groups and 12 comparable groups fed stilbestrol gave a correlation coefficient of 0.87

which was significant ($P<.01$). From the regression line, it appears that stilbestrol had no influence upon carcass fatness with rations containing 56 Calories per pound per percent of ration protein. Conversely, stilbestrol brought about substantial changes in carcass fatness when this ratio was either below or above this figure. The greatest increase in fat in the carcass resulting from stilbestrol was about 2.5%; the Calorie: protein ratio of the ration was about 75. Similarly, the greatest decrease was about 3.7%; the Calorie: protein ratio of the ration being about 30. Expressing these changes in fat content another way, namely, as a percentage change in the total separable fat rather than as the change in percentage of separable fat, the influence of stilbestrol upon carcass fatness became more evident. Upon this basis stilbestrol in the ration of highest Calorie to protein ratio increased carcass fatness by about 10%, whereas with the lowest Calorie to protein ratio it decreased carcass fatness by about 15%. This would suggest that the feeding of stilbestrol to lambs could result in either an increase or a decrease in the amount of separable fat, depending upon the Calorie: protein ratio of the fattening ration. If one considers only the high-energy rations shown in figure 1, the above trend appears even more striking. Changes in the fat content of the longissimus dorsi M. were similar but less consistent with respect to stilbestrol feeding.

The weight of kidney fat and thickness of fat cover over the twelfth rib showed significant treatment changes which were similar to the changes noted with respect to separable fat measurements of the carcass rack. The rib eye areas were increased from 1.98 sq. in. on the low energy ration to 2.11 sq. in. on the high energy rations. Also ration protein levels increased rib eye areas a similar amount; however, a lesser increase was noted from the stilbestrol additions to the rations.

Other Observations. Stilbestrol increased the weights of the seminal vesicle and bulbo-urethral glands. It slightly increased the weight of the adrenal glands, especially at the high level. The anterior pituitary weights were significantly affected by the levels of protein ($P=0.03$) and by the energy-stilbestrol interaction ($P=0.03$). The response to the protein levels was quadratic. The reason for this type of response is not clear. Stilbestrol increased the weight of the anterior pituitary ($P=0.08$); however, this increase took place primarily on the high energy ration, which accounts for the significant interaction between energy and stilbestrol levels ($P=0.03$). It was also on the high energy ration that stilbestrol resulted in the largest stimulation of liveweight gains. The weight of the thyroid gland appeared to be relatively unaffected by treatment other than the level of protein ($P=0.07$). As the level of protein was increased, the size of the thyroid was increased. Kidney weight was markedly increased by the increasing levels of protein ($P<0.01$) and slightly increased by stilbestrol ($P=0.05$). The kidneys from the lambs on the high energy ration were larger than those from the lambs on the low energy ration ($P=0.06$).

Liver weights were increased slightly but significantly by the increasing levels of stilbestrol, protein and energy.

Blood serum amino acid nitrogen was generally decreased by the increasing levels of stilbestrol (5.34, 5.28 and 5.00 mg. per 100 ml., for the 0, 0.3 and 0.6 mg. levels, respectively). The low energy-17% protein ration gave a notable exception. It should be recalled that stilbestrol also did not elicit a gain stimulation on this particular energy-protein combination. In general, the decrease in serum amino nitrogen was more uniform on the high energy than on the low energy rations. The higher protein level appeared to increase the serum amino nitrogen. Serum glucose concentrations were consistently increased by stilbestrol ($P<0.01$). Average values for the 0, 0.3 and 0.6 mg. levels were 75.9, 86.2 and 86.1 mg. per 100 ml., respectively. The high energy rations resulted in higher serum glucose values (80.2 and 85.2 mg. per 100 ml., respectively). The higher protein levels (9, 13 and 17%) appeared to reduce ($P=0.02$) the serum glucose values (85.3, 84.4 and 78.5 mg. per 100 ml., respectively).

Discussion

Since stilbestrol is widely recognized as a gain-stimulating material and is presumably not a nutrient, it would seem logical to assume from a nutritional standpoint that it should exert its maximum usefulness in rations which are nutritionally adequate; also, from the body physiology standpoint, that stilbestrol should increase rather than decrease the daily needs for all nutrients concerned in growth processes. The results obtained in this experiment do not in any way conflict with this general hypothesis. Instead, the results substantiate it in that stilbestrol appeared to stimulate maximum increase in liveweight gains with all the rations tested within the limits of their nutritional adequacy. The nutritional adequacy of all rations tested was essentially complete with the exception of energy for maximum fattening in the series of low energy rations. Even the series of low protein rations had 90% of the recommended nutrient allowances for growing and fattening lambs (N.R.C., 1950) which probably was sufficient to meet actual protein needs. Therefore, it was not surprising that in this experiment stilbestrol stimulated liveweight gains in the lambs and improved feed efficiency with five of the six rations tested.

Probably the most important result in this experiment was the combined influence of protein and energy in altering the effect or lack of effect of stilbestrol upon the degree of fatness in the carcasses of the lambs. It will be recalled that stilbestrol increased the fatness of the lamb carcasses only when an adequate but relatively low level of protein was fed in a high energy ration and that this same influence did not occur with other rations containing just as much available net energy but higher levels of protein. This would suggest that stilbestrol either directly or indirectly is capable

of bringing about a greater or lesser conversion of ration energy into body fat depending upon the ratio of ration protein to ration energy rather than upon the total net energy of the ration. The physiological basis of this influence of stilbestrol is not known but probably it is concerned with altered body requirements for both energy and protein brought about by stilbestrol administration. This in turn may be due to an altered rate and/or pathway of metabolic reactions.

From the stand point of general livestock production, the results obtained in this experiment would seem to have special application if similar results can be uniformly achieved in cattle and lamb feeding practice. Where additional fatness is a desirable feature in meat carcasses from these animals, this might be obtained by adding stilbestrol to a ration having a high Calorie to protein ratio; and additional leanness by adding it to a ration having a relatively low Calorie to protein ratio.

The results of the endocrine and blood analyses in this experiment would tend to indicate that stilbestrol possesses a growth hormone-like action or stimulates the production of growth hormone in the animal body. Increased anterior pituitary weight and serum glucose, and the decreased serum amino acid level obtained are each believed to be indicative of this type of response.

The allotting of the lambs on the basis of initial finish and body weight significantly reduced the error mean square in the analyses of variance of daily gain, feed efficiency, live grade, separable lean and fat, thickness of fat cover, anterior pituitary weights, kidney weights, and serum glucose values. Of the two, the effect of initial finish was much more important.

Summary

Eighteen rations differing with respect to amount of protein, stilbestrol, and net energy were fed in a 3x3x2 factorially designed experiment to 144 growing and fattening lambs. Individual feed records and liveweight gains of the lambs were recorded, and at the termination of the feeding period the animals were slaughtered and detailed physical and chemical measurements were made upon the carcasses together with measurements of certain blood constituents and selected endocrine glands.

Stilbestrol stimulated liveweight gains and improved feed conversion in most instances. It gave somewhat more stimulation with high energy and high protein rations than with low energy and low protein rations. Carcass fatness was unaltered by stilbestrol feeding when the Calorie-protein ratio of the rations was near 56. With rations of higher Calorie-protein ratios, stilbestrol increased carcass fatness and with rations of lower ratios it resulted in leaner carcasses with less fat. Dressing percent was increased slightly by the high protein rations, decreased slightly by stilbestrol, and increased substantially by the high energy rations. In general, the lambs fed the high energy and the high protein rations had higher live and car-

cass grades. Stilbestrol did not affect live or carcass grades. The weights of the anterior pituitary, kidneys, liver and secondary sex glands were all increased by stilbestrol feeding. The high energy rations produced lambs with heavier kidneys and livers. The weights of the thyroids, kidneys and liver were increased as the level of protein was increased. Serum glucose was significantly increased by stilbestrol and by the high energy rations, and reduced by the high protein rations. Serum amino nitrogen was not affected appreciably by the various treatments; however, it was somewhat lower when stilbestrol was fed and higher when the high protein rations were fed.

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CHLORTETRACYCLINE AND TETRA ALKYLAMMONIUM STEARATE IN LAMB RATIONS[1]

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MANY commercial lamb feeders incorporate antibiotics in their lamb rations as a means of increasing the rate of gain and reducing the incidence of enterotoxemia. Kunkel *et al.* (1956) and Johnson *et al.* (1956) reported a material reduction in the incidence of enterotoxemia in fattening lambs that were fed antibiotics in conjunction with a fattening ration. Hanke *et al.* (1957) and Hatfield *et al.* (1954) reported an increase in average daily gain and feed efficiency in fattening lambs receiving chlortetracycline in their rations. However, Keith *et al.* (1955) reported no increase in average daily gain by lambs receiving chlortetracycline, and Jordan *et al.* (1956) reported an inconsistant response between trials from chlortetracycline when fed to fattening lambs.

Recently a feed additive, tetra alkylammonium stearate, referred to as a chemobiotic and marketed under the name of "Dynafac", has been suggested as a means of preventing enterotoxemia and increasing the rate of gain and feed efficiency of fattening lambs. Beeson *et al.* (1957) reported a slight, though not significant, increase in rate of gain when tetra alkylammonium stearate, "Dynafac", was fed to heifer calves receiving high moisture ground ear corn, but no effect on gains when tetra alkylammonium stearate was added to a grain ration consisting of regular ground ear corn.

The object of this experiment was to study the effects of chlortetracycline and tetra alkylammonium stearate on rate of gain, feed consumption, scouring and enterotoxemia of fattening lambs.

Materials and Methods

Two trials involving 185 good quality native feeder lambs were conducted during the winter and spring months of 1956–57. In both trials the lambs were sheared before being placed on experiment and all the lambs were hand-fed a fattening ration of shelled corn, brome hay, protein supplement, trace mineralized salt and bone meal. The lambs were individually weighed every 14 days. Analysis of variance (Snedecor, 1946) was the method used for the statistical analysis of the data.

In the first trial, 70-lb. feeder lambs were fed, in addition to their hay and grain, the following protein supplements: Lots 1, 2 and 3, received soybean oil meal and Lots 4, 5 and 6, received a mixture of feather meal and

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ground corn. Protein levels were equalized between Lots. To these two protein supplements were added the following treatments: Lots 1 and 6, control; Lots 2 and 4, 200 mg. of tetra alkylammonium stearate per lamb daily; and Lots 3 and 5, 40 mg. of chlortetracycline per lamb daily.

In the second trial, 80-lb. feeder lambs were used as test animals and soybean oil meal was the protein supplement fed in all Lots. The following

TABLE 1. CHLORTETRACYCLINE AND TETRA ALKYLAMMONIUM STEARATE WITH SOYBEAN OIL MEAL OR FEATHER MEAL IN LAMB RATIONS—TRIAL 1

Protein supplement	Soybean oil meal			Feather meal		
Lot number	1	2	3	4	5	6
Treament[a]	Control	T	C	T	C	Control
No. lambs[b]	14	14	16	15	16	14
Initial av. wt., lb.	70.3	71.8	71.0	70.4	70.8	69.1
Final av. wt., lb.	103.7	103.6	103.8	101.9	103.3	100.5
Gain per lamb, lb.	33.4	31.8	32.8	31.5	32.5	31.4
No. days fed	70	70	70	70	70	70
Av. daily gain, lb.	.48	.45	.47	.45	.46	.45
Av. daily feed consumption						
Grain, lb.	1.44	1.41	1.47	1.43	1.52	1.38
Hay, lb.	1.95	1.91	1.83	1.75	1.91	1.85
Protein supplement, lb.	.20	.20	.20	.20	.20	.20
Feed per 100 lb. gain						
Grain, lb.	301.9	310.6	313.4	317.8	327.6	307.3
Hay, lb.	408.8	420.7	390.2	388.9	411.6	407.5
Protein supplement, lb.	41.9	44.1	42.6	44.4	43.1	44.5

[a] Treatments: Lot 1, soybean oil meal; lot 2, soybean oil meal plus tetra alkylammonium stearate; lot 3, soybean oil meal plus chlortetracycline; lot 4, feather meal plus tetra alkylammonium stearate; lot 5, feather meal plus chlortetracycline; and lot 6, feather meal. All lambs were hand-fed a fattening ration of shelled corn and brome hay.

[b] Death due to enterotoxemia: 1 lamb in lot 2; 1 lamb in lot 4; 2 lambs in lot 6.

replicated treatments were employed: Lots 1 and 4, control; Lots 2 and 5, 40 mg. chlortetracycline per lamb daily; and Lots 3 and 6, 200 mg. of tetra alkylammonium stearate per lamb daily. The production data from both trials are presented in tables 1 and 2.

Results and Discussion

Rate of Gain. In the first trial the addition of chlortetracycline or the addition of tetra alkylammonium stearate failed to have any real effect on rate of gain. This was true whether the additives were added to a ration containing soybean oil meal or feather meal as a source of supplemental protein. Average daily gains in the Lots were as follows: Control lambs. Lots 1 and 6, 0.477 and .449 lb. per lamb daily; lambs fed tetra alkylam-

monium stearate, Lots 2 and 4, 0.45 and .450 lb. per lamb daily; and chlortetracycline-fed lambs, Lots 3 and 5, 0.469 and 0.464 lb. per lamb daily.

In the first replica of the second trial, the control lambs (Lot 1) gained 0.03 lb. faster than the lambs receiving chlortetracycline supplement. However, the rates of gain of the control lambs and the lambs receiving tetra alkylammonium stearate (Lot 3) were almost identical (Lot 1, 0.0669; Lot 2, 0.639 and Lot 3, 0.664).

TABLE 2. CHLORTETRACYCLINE AND TETRA ALKYLAMMONIUM STEARATE IN FATTENING LAMB RATIONS—TRIAL 2

	Replica 1			Replica 2		
Lot number	1	2	3	4	5	6
Treatment[a]	Control	C	T	Control	C	T
No. lambs[b]	15	16	15	16	15	14
Initial av. wt., lb.	80.1	79.9	78.7	80.0	79.6	80.0
Final av. wt., lb.	119.6	117.6	117.9	116.2	116.9	118.4
Gain per lamb, lb.	39.5	37.7	39.2	36.2	37.3	38.4
No. days fed	59	59	59	59	59	59
Av. daily gain, lb.	.67	.64	.66	.61	.63	.65
Av. daily feed consumption						
Grain, lb.	1.85	1.85	1.86	1.77	1.85	1.86
Hay, lb.	1.72	1.68	1.59	1.56	1.75	1.62
Protein supplement, lb.	.2	.2	.2	.2	.2	.2
Feed per 100 lb. gain						
Grain, lb.	276.5	289.5	280.1	288.2	292.7	285.7
Hay, lb.	257.1	262.9	239.4	254.0	276.9	248.8
Protein supplement, lb.	29.1	31.3	30.1	32.5	31.6	30.7

[a] Treatments: Lot 1, basal; lot 2, basal plus chlortetracycline; lot 3, basal plus tetra alkylammonium stearate; lot 4, replica of lot 1; lot 5, replica of lot 2; lot 6, replica of lot 3. All lambs were hand-fed a fattening ration of shelled corn, brome hay and soybean oil meal.

[b] Death due to enterotoxemia: 1 lamb lot 5.

In the second replica, the control lambs (Lot 4) gained slightly slower than either the lambs receiving chlortetracycline (Lot 5) or tetra alkylammonium stearate (Lot 6). Rate of gain of the three Lots were: Lot 4, 0.614; Lot 5, 0.632 and Lot 6, 0.651. The slower rate of gain made by the control lambs in the second replica may be due to the lower grain and hay consumption of the control lambs. Contrary to the first trial, the lambs in the second trial that received tetra alkylammonium stearate gained slightly faster than the lambs receiving chlortetracycline. However, when the data were analyzed statistically no significant difference between treatments, replications or interaction of treatment and replication existed. The excellent gains made by the control lambs in both trials suggest that the level of disease present in the lambs was low. This may account for the lack of response to either of the feed additives.

Feed Consumption. The grain and hay consumption by the lambs fed in the first trial was quite similar, however, the lambs in Lots 3 and 5 (chlortetracycline) consumed slightly more grain than the other Lots. The least amount of hay was eaten when feather meal was used as the protein supplement in conjunction with tetra alkylammonium stearate (Lot 4).

There were little differences in feed consumption existing in the second trial. There was a tendency, however, for the lambs receiving tetra alkylammonium stearate (Lots 3 and 6) to consume slightly less roughage than the lambs receiving chlortetracycline (Lots 2 and 5). The slight and inconsistant differences suggest that neither chlortetracycline nor tetra alkylammonium stearate had any real effect on feed consumption.

Other Factors. During the first two weeks of the first trial, lambs receiving tetra alkylammonium stearate had a tendency to bloat. This was true in both Lots 2 and 4, and appeared in about 90% of the lambs. This condition disappeared after about two weeks time and did not reappear. Furthermore, it did not occur in any of the lambs receiving tetra alkylammonium stearate in the second trial. During the first three weeks of the first trial, all of the lambs developed scours. Initially this condition was equally troublesome in all Lots but cleared up somewhat sooner in the Lots receiving chlortetracycline.

The lambs were not vaccinated and were kept on as large a feed intake as possible so as to observe the effects of either chlortetracycline or tetra alkylammonium stearate on enterotoxemia. In the first trial two lambs receiving tetra alkylammonium stearate and two control lambs died of enterotoxemia. In the second trial one lamb receiving chlortetracycline died of enterotoxemia.

Summary

Two trials were conducted to determine the effect of chlortetracycline and tetra alkylammonium stearate on fattening lambs. Neither chlortetracycline nor tetra alkylammonium stearate significantly increased rate of gain, feed consumption or feed efficiency. Death loss due to enterotoxemia occurred in lambs receiving chlortetracycline or tetra alkylammonium stearate.

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THE EFFECTS OF DIETHYLSTILBESTROL, TESTOSTERONE AND RESERPINE ON GROWTH AND CARCASS GRADE OF LAMBS [1, 2]

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IT has been shown repeatedly that a variety of estrogens produce an increase in rate of gain and feed efficiency in lambs but that carcass quality, as reflected by Federal grading standards, is generally reduced. There has been some evidence that carcass grade has been improved by both antibiotic and androgen administration. There is also evidence that in mentally retarded patients, the use of certain tranquilizing agents is followed by an improvement in general well-being. For these reasons, it was decided to study the effects of combinations of hormonal substances, chlortetracycline and reserpine on the growth of lambs.

Materials and Methods

Two experiments involving a total of 372 ewe and wether western feeder lambs were conducted. Experiment I was performed during the 84-day period between October 18, 1955, and January 10, 1956. All lambs were fed a pelleted ration composed of 50% dehydrated alfalfa meal, 45% ground yellow corn and 5% soybean oil meal; this was fed free choice. Mixed legume-grass hay was available *ad libitum,* and a mineral mixture of two parts steamed bone meal and one part iodized salt was constantly available. Twelve lots of 20 lambs each were used. The lot treatments are shown in table 1.

TABLE 1. LOT TREATMENTS IN EXPERIMENT I

No antibiotic		Chlortetracycline, 6 mg. per lb. ration
Lot I	Control	Lot II
Lot III	6 mg. DES implant [a]	Lot IV
Lot V	1 mg. DES per lb. ration	Lot VI
Lot VII	1 mg. DES per lb. ration for first 42 days only	Lot VIII
Lot IX	.25 mg. of reserpine per lb. ration	Lot X
Lot XI	.50 mg. of reserpine per lb. ration	Lot XII

[a] Diethylstilbestrol.

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[2] Supported in part by a grant from Chas. Pfizer and Company, Inc. The reserpine was supplied by Merck and Company.

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Because the lambs implanted with diethylstilbestrol (DES) gained more rapidly than the others, it was necessary to market them after 70 days of treatment (Lots III and IV), especially since those in Lot III weighed approximately 112 lb. at that time. The remaining lots were slaughtered at the end of 84 days when weighing approximately 100 lb. As shown in table 3, a total of five lambs did not complete the experiment. Lambs were removed from Lots I and VIII for general unthriftiness. There was a death in Lot II, a case of generalized edema in Lot IV and one lamb with chronic bloat in Lot VI.

Experiment II was carried out between November 1, 1956, and January 7, 1957—a period of 67 days. All lambs were fed *ad libitum* a pelleted complete ration of the following composition:

Ingredient	Percent
Ground corn cobs	39.50
Ground yellow corn	32.00
Dehydrated alfalfa meal	20.00
Soybean oil meal	7.00
Steamed bone meal	1.00
Salt plus cobalt [a]	0.50
Vitamin A and D concentrate [b]	0.075

[a] One ounce cobalt carbonate per 100 lb. of salt.
[b] Supplied 1,700 U.S.P. units of vitamin A and 212 U.S.P. units of vitamin D per pound of ration.

A mineral mixture of two parts steamed bone meal and one part iodized salt was available separately. Six lots of 23 lambs each were used. The treatments are shown in table 2.

All hormonal substances were implanted subcutaneously in the ear. At the conclusion of each experiment, the lambs were slaughtered in Indianapolis. On the day following slaughter, the carcass of each lamb was graded according to U.S.D.A. standards by a Federal grader. The lambs were distributed in the cooler at random and treatments were unknown to the grader.

Results

Growth Rate. The data on average daily gains and total gains in experiment I are summarized in table 3. Since the lambs in Lots III and IV

TABLE 2. LOT TREATMENTS IN EXPERIMENT II

Lot I	Control
Lot II	Implanted with 3 mg. DES [a] pellet
Lot III	Implanted with 6 mg. DES pellet
Lot IV	Implanted with two 10 mg. TP [b] pellets
Lot V	Implanted with 3 mg. DES pellet and two 10 mg. TP pellets
Lot VI	Implanted with 6 mg. DES pellet and two 10 mg. TP pellets

[a] Diethylstilbestrol.
[b] Testosterone propionate.

TABLE 3. THE EFFECT OF DIETHYLSTILBESTROL, CHLORTETRACYCLINE AND RESERPINE ON LAMBS (84 DAYS)[a]

Item	I Control	II Antibiotic	III DES implant	IV DES implant antibiotic	V DES orally	VI DES orally antibiotic	VII DES orally 42 days	VIII DES orally 42 days antibiotic	IX 0.25 mg. reserpine	X 0.25 mg. reserpine antibiotic	XI 0.50 mg. reserpine	XII 0.50 mg. reserpine antibiotic
No. of lambs	19	19	20	18	20	19	20	19	20	20	20	20
Av. initial wt., lb.	76	76	76	76	75	75	75	74	75	76	75	75
Av. final wt., lb.	101	101	112	102	105	103	102	99	103	103	97	94
Total gain, lb.	25	25	36	26	30	28	27	25	28	27	22	19
Av. daily gain, lb.	.29	.29	.51	.37	.36	.33	.32	.30	.33	.32	.26	.22
Daily feed per lamb												
pelleted ration, lb.	2.82	2.57	3.09	2.50	2.70	2.58	2.78	2.49	2.69	2.65	2.33	2.12
hay, lb.	.20	.19	.38	.24	.19	.19	.19	.19	.19	.19	.19	.19
Feed per lb. of gain												
pelleted ration, lb.	9.43	8.55	6.00	6.63	7.62	7.87	8.67	8.43	8.18	8.14	8.79	9.52
hay, lb.	.67	.65	.70	.62	.54	.59	.60	.65	.58	.60	.72	.86
Federal carcass grades												
no. high choice		1								1		
no. average choice	4	4	2		6	4	4	5	6	5	3	8
no. low choice	9	4	4		4	6	8	8	5	7	7	6
no. high good	6	7	11	4	7	7	4	3	6	5	6	5
no. average good		2	2	9	2	1	2	1	2	1	2	1
no. low good				2	1		2		1	1		
no. high utility		1	1	1		1		2			2	
Average numerical grade[c]	6.89	6.52	6.47	5.0[b]	6.60	6.52	6.50	6.57	6.65	6.85	6.25	7.05

[a] The lambs in Lots III and IV were marketed at 70 days.
[b] The carcasses of two lambs were misplaced in the coolers.
[c] Numerical designation of grades was as follows: high choice, 9; average choice, 8; low choice, 7; high good, 6; average good, 5; low good, 4; high utility, 3.

were slaughtered two weeks prior to the others, comparisons were made on the basis of average daily gain. An analysis of variance, in which each lot was compared separately with the control group, showed that average daily gain per lamb was highly significantly increased in Lot III by the implantation of 6 mg. of DES ($P<0.01$) and was significantly increased ($P<0.05$) by the implantation of 6 mg. of DES and the feeding of chlortetracycline (Lot IV) or the feeding of 1 mg. of DES per pound of ration (Lot V). The addition of chlortetracycline alone had no effect on

TABLE 4. THE EFFECT OF ESTROGEN AND ANDROGEN ON LAMBS (67 DAYS)

	I	II	III	IV	V	VI
	Control	3 mg. DES implant	6 mg. DES implant	20 mg. TP implant	3 mg. DES 20 mg. TP implants	6 mg. DES 20 mg. TP implants
No. of lambs	23	23	23	23	23	23
Av. initial wt., lb.	73.0	75.0	76.0	75.0	75.0	76.0
Av. final wt., lb.	105.0	113.0	115.0	111.0	116.0	118.0
Total gain, lb.	32.0	38.0	39.0	36.0	41.0	42.0
Av. daily gain, lb.	.48	.57	.58	.54	.61	.62
Daily feed per lamb						
Complete pelleted ration, lb.	3.88	4.13	4.13	4.09	4.14	4.17
Feed per lb. of gain						
Pelleted ration, lb.	8.00	7.24	7.11	7.71	6.69	6.65
Federal carcass grades						
No. high choice						
No. average choice	4	4	1	2	1	2
No. low choice	12	5	3	15	1	4
No. high good	2	7	6	3	13	8
No. average good	4	6	9	2	8	8
No. low good	1	1	3	1		1
No. high utility			1			
Average numerical grade[a]	6.60	6.21	5.40	6.65	5.78	5.91

[a] Numerical designation of grades are as follows: high choice 9, average choice 8, low choice 7, high good 6, average good 5, low good 4, high utility 3.

rate of gain; but in each instance when it was fed in combination with DES or reserpine, there was a reduction in daily gain. The feeding of reserpine at a level of 0.25 mg. per pound of ration did not increase gains, significantly, and at the level of 0.50 mg. per pound of ration, there was a reduction in rate of gain. The higher level of reserpine in combination with antibiotic significantly reduced gain ($P<0.05$). It was apparent that the lambs receiving the higher level of reserpine were lethargic, and their daily feed intake was lower than any other group.

In experiment II (table 4) there was a marked improvement in average daily gain in those lambs implanted with either 3 or 6 mg. of DES alone or in combination with a 20-mg. implantation of testosterone propionate (TP). The differences were significant at the 1% level of probability except in the case of Lot II in which the probability was not quite 1%. The DES and TP combination produced the best gains, but the differences

between DES alone and the combination were not statistically significant.

Feed Consumption and Efficiency. There were no lot replications in either trial; thus, statistical treatment of the feed data was not attempted. As in several previous experiments, there was an apparent improvement in gross feed efficiency in all groups of lambs which received DES by implantation or in the feed, or in combination with TP. In general, the feeding of chlortetracycline at a level of 6 mg. per pound of ration (table 3) reduced feed efficiency when fed in combination with DES or the high level of reserpine. The animals which were fed 0.25 mg. reserpine per pound of ration required less feed per unit of gain than the controls but were not as efficient as the DES-treated sheep. There was a marked reduction in daily feed consumption and rate of gain in the lambs which were fed 0.50 mg. of reserpine and 6 mg. of antibiotic per pound of ration (table 3).

In experiment II (table 4) the implantation of DES alone or in combination with TP, or TP alone, consistently improved gross feed efficiency. The combination was more effective than either substance alone. The maximum saving in feed (17%) occurred in the lambs which received 6 mg. of DES and 20 mg. of TP.

Carcass Studies. A summary of the various carcass grades is given in tables 3 and 4. The Federal graders made three divisions of both the choice and good grades; namely, high, average and low. As previously reported by this Station and other workers, there has been consistant reduction in carcass grade when DES was administered. The reduction in grade appears to be related to the amount of estrogen absorbed. As shown in table 4, the carcass grades of the sheep receiving a 6-mg. DES pellet were lower than those which received 3 mg.; and in experiment I, the lambs fed 1 mg. DES per pound of ration did not have as high carcass grades as the controls. It is interesting to observe that the lambs which received reserpine in combination with chlortetracycline had carcasses equal to or superior to the controls. For purposes of comparison, numerical values were assigned to the carcass grades as shown in tables 3 and 4. The average numerical score is shown in tables 3 and 4. As discussed by Andrews *et al.* (1956), techniques for statistically evaluating carcass grade data leave much to be desired. However, an analysis of variance of the numerical scores in experiment II indicates that the carcasses of the controls and the TP-treated lambs (Lot 4) were superior to all others and that the implantation of a single 6-mg. pellet of DES significantly reduced carcass grade ($P<0.01$).

Discussion

As summerized by Andrews *et al.* (1949–1956), Jordan *et al.* (1950–1956), Clegg and Cole (1954), Henneman *et al.* (1957), Taylor *et al.* (1957) and others, the administration of estrogens either by implantation or orally, or combinations of estrogens and progesterone, have consistently

improved rate of gain and feed efficiency in a variety of experiments at a large number of experiment stations. Depending on the level and duration of time of hormone administration, and the length of the feeding period and market weight of the lambs, there have been instances in which carcass grade has been closely similar to the untreated sheep; but in general, carcass grade has been lowered. As reviewed by the authors mentioned, the reduction in grade appears to be largely the result of decreased fat deposition.

The effects of androgenic substances on fattening lambs are neither as well-known nor as consistent as is the case with estrogens. As early as 1949 Andrews *et al.* reported that the subcutaneous implantation of a 10-mg. pellet of TP significantly increased rate of gain; in the present experiment, although there was a slight increase in rate of gain at the 20-mg. level, the difference was not significant. However, the carcass grades of the TP-treated lambs were distinctly superior to the estrogen-treated animals. In two subsequent experiments, Means *et al.* (1953) produced a highly significant increase in rate of gain ($P<0.01$) following the implantation of 30 mg. of TP. In one trial (Means *et al.*), carcass quality was equal to the controls; and in the other, the controls were superior. The feeding of methyl testosterone at a level of 25 mg. per lamb daily did not improve rate of gain or carcass quality (Andrews *et al.*, 1956). O'Mary *et al.* (1952) concluded that the implantation of a 15-mg. pellet of testosterone improved gain but not significantly and that its effects on growth, fattening and the carcass tended to be qualitatively similar to DES but not as marked. Henneman *et al.* (1957) reported that the implantation of lambs with 60 mg. of testosterone, or 100 mg. of delta 4 androstene 3,17 dione, resulted in no improvement in gain, feed efficiency or carcass grade. Taylor *et al.* (1957) found that the daily injection of TP improved gain in one of three trials with ewe lambs and that implantation with 30 mg. of TP was ineffective. The feeding of methyl testosterone produced an increase in rate of gain in one of three trials.

Research with androgens has been limited by the higher cost when compared to the relatively cheap stilbenes and in part because of a scarcity of androgens with high oral activity. The increased availability of anabolic androgens such as 19 nortestosterone and its derivatives should result in more experimentation with androgenic substances.

Summary

Two experiments involving a total of 372 ewe and wether lambs were conducted. Rate of gain was significantly increased by the implantation of 3 or 6 mg. pellets of diethylstilbestrol alone or in combination with an implantation of 20 mg. of testosterone propionate. The estrogen-androgen combination produced the most rapid gains, but the improvement was not statistically significant in comparison with diethylstilbestrol. The feeding

of DES at a level of 1 mg. per pound of ration improved rate of gain but not as much as when DES was implanted. The oral administration of reserpine at a level of 0.25 mg. per pound of ration increased gain slightly but decreased growth when fed at a level of 0.50 mg. per pound and significantly reduced growth when fed at the 0.50 mg. level in combination with chlortetracycline. There was an apparent improvement in gross feed efficiency in all groups of lambs that received DES in the feed, by implantation or in combination with testosterone propionate. The maximum feed saving occurred in the lambs which were implanted with 6 mg. of DES and 20 mg. of TP.

Carcass grades were consistently reduced by any form of DES administration, and the reduction appeared to be related to the amount of hormone used. The lambs receiving testosterone propionate alone had carcasses equal to the controls. It is regarded as an interesting observation that the combination of reserpine and chlortetracycline produced carcasses equal or superior to the controls although rate of gain was reduced at the higher reserpine level.

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THE COMPARATIVE EFFECTIVENESS OF ORAL AND SUBCUTANEOUS IMPLANTATION OF DIETHYLSTILBESTROL IN COMBINATION WITH CHLORTETRACYCLINE [1]

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SOME of the earliest research concerned with the use of diethylstilbestrol implants in beef cattle was reported from this Station (Dinusson *et al.*, 1950) in which the implantation of either 42 or 48 mg. diethylstilbestrol per heifer resulted in significantly increased rates of gain. The levels of diethylstilbestrol implants used for steers at the same Station (Andrews *et al.*, 1950, 1951) 60 mg. and 120 mg. per animal resulted in significantly increased gains. However, no attempt was made to establish either minimal or optimal levels of the implants. Burroughs *et al.* (1954) reported that diethylstilbestrol administered daily *per os* was effective in stimulating the rates of gain of beef cattle. The purpose of the research reported in this paper was to study: (1) various levels of diethylstilbestrol implants ranging from 12 to 48 mg. for steer calves; (2) the comparative efficacy of diethylstilbestrol pellets (implanted under the skin at the base of the ear) with the daily oral feeding of diethylstilbestrol; and (3) the effect of adding an antibiotic with each of the above treatments.

Materials and Methods

Seventy-two steer calves, averaging 513 lb. liveweight at the start were divided into 12 lots of 6 steers each, on the basis of liveweight and apparent thrift. All 12 lots were fed a fattening ration of a full feed of ground ear corn, 2 lb. Purdue cattle supplement A, 15 lb. corn silage, and free choice mineral mixture of 2 bone meal: 1 salt, plus loose salt, through July 10, when the supply of corn silage was depleted. Starting July 10, the ration consisted of a full feed of ground ear corn, free choice ground corn cobs, 3.50 lb. Purdue cattle supplement A, and free choice minerals as indicated above. The treatments are shown in table 1.

Pellets containing 12 mg. of diethylstilbestrol per pellet were implanted subcutaneously in the ear on the first day of the experiment. Dosages of 12, 24, 36 and 48 mg. per steer were used.

Ten grams of diethylstilbestrol were dissolved in 500 ml. of corn oil and pre-mixed with 10 lb. of soybean oil meal before mixing in a ton of supple-

[1] Contributions from the Department of Animal Husbandry, Journal Paper No. 1168.
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ment A for Lots II and VIII. Since the cattle fed diethylstilbestrol consumed 2 lb. of supplement A per day, each pound of supplement A contained 5 mg. diethylstilbestrol, and thus each steer received 10 mg. diethylstilbestrol daily in Lots II and VIII. When the ration was changed from corn silage to corn cobs as a source of roughage, 2.0 lb. of supplement A containing 5 mg. of diethylstilbestrol per pound was fed plus 1.5 lb. supplement A without diethylstilbestrol.

Chlortetracycline was supplied as Aurofac 2A which contained 3.6 gm. chlortetracycline per pound. Therefore, each ton of supplement A contain-

TABLE 1. TREATMENTS [a]

Lot no.	Diethylstilbestrol		Chlortetracycline fed per steer daily
	Fed per steer daily	Implanted under skin, one time	
	mg.	mg.	mg.
I	0	0	0
II	10	0	0
III	0	12	0
IV	0	24	0
V	0	36	0
VI	0	48	0
VII	0	0	80
VIII	10	0	80
IX	0	12	80
X	0	24	80
XI	0	36	80
XII	0	48	80

[a] Formula for Purdue Supplement A, 1,000 lb. mix: soybean oil meal, 650.5 lb.; molasses, 140 lb.; dehydrated alfalfa meal, 140 lb.; steamed bone meal, 52 lb.; salt with cobalt (one ounce cobalt sulfate per 100 lb. salt), 17 lb.; Vitamin A and D concentrate (4,540,000 U.S.P. units of A and 567,500 U.S.P. units of D per pound), 0.5 lb.

ing chlortetracycline was fortified with 22.2 lb. of Aurofac 2A which provided 80 gm. of chlortetracycline. Thus, each pound of supplement A contained 40 mg. of chlortetracycline. When the ration was changed from corn silage to ground corn cobs, as a source of roughage, 2.0 lb. of supplement A containing 40 mg. chlortetracycline per pound was fed, plus 1.5 lb. supplement A without chlortetracycline.

At the completion of the trial, the cattle were followed through marketing and slaughtered where information on the effect of treatment on shrink in-transit, dressing percentage, and U. S. Federal carcass grades was obtained.

Results

Effect of Level of Diethylstilbestrol Implantation. The data summarized in table 2 are for a period of 233 days. It is apparent that for this interval

of time 12 and 24 mg. diethylstilbestrol were not as effective as the 36 and 48 mg. levels. It appears that a dosage level of 36 mg. per steer is near the optimum for cattle fattened in drylot for a period in excess of 7 months. At the end of the second month of treatment the cattle which

TABLE 2. ORAL DIETHYLSTILBESTROL (DES) VS. LEVELS OF IMPLANTED DIETHYLSTILBESTROL, WITH AND WITHOUT CHLORTETRACYCLINE (CTC), INITIAL WT. 513 LB.

(FEBRUARY 7–SEPTEMBER 17, 1956. 233 DAYS)

	Feed per 100 lb. gain, lb.[a]			
	Gr. ear corn	Suppl. A	Roughage[b]	Daily gain
				lb.
Lot I—Control	471	105	247	2.19
Lot II—10 mg. DES orally, per day	434	91	222	2.52*
Lot III—12 mg. DES implant	467	100	240	2.31
Lot IV—24 mg. DES implant	475	98	240	2.36
Lot V—36 mg. DES implant	437	89	221	2.59*
Lot VI—48 mg. DES implant	458	93	231	2.47*
Lot VII—chlortetracycline 80 mg. per day	453	99	235	2.31
Lot VIII—same as II, plus 80 mg. CTC	427	87	215	2.65*
Lot IX—same as III, plus 80 mg. CTC	447	89	224	2.59*
Lot X—same as IV, plus 80 mg. CTC	469	93	234	2.47*
Lot XI—same as V, plus 80 mg. CTC	440	88	220	2.63*
Lot XII—same as VI, plus 80 mg. CTC	443	88	221	2.61*

[a] Daily ration consisted of: ground ear corn, full feed; corn silage, 15.0 lb.; Supplement A, 2.0 lb.; minerals, free choice.

[b] Corn silage was fed from February 7 to July 10. Ground corn cobs were fed from July 10 to September 17. Roughage was calculated on a dry matter basis.

* Gained significantly more than Lot I or Lot VII.

received 24 mg. of diethylstilbestrol were gaining as rapidly as any group, but after the third month those which received either 36 or 48 mg. gained most rapidly. At the end of the fourth month the cattle which received a 12 mg. implant had an average daily gain of 2.74 lb. per day but rate of gain declined beyond this time.

Oral vs. Implantation as Methods of Administering Diethlystilbestrol. The administration of diethylstilbestrol by the implantation of pellets

is an efficient method (table 3). The steers implanted with 36 mg. diethylstilbestrol (Lot V) gained slightly more rapidly than those fed their diethylstilbestrol (Lot II). Furthermore, the steers fed 10 mg. of

TABLE 3. ORAL VS. IMPLANTED DIETHYLSTILBESTROL FOR FATTENING CALVES

	No DES Lots I+VII	10 mg. DES orally, daily Lots II+VIII	36 mg. DES implanted once under skin Lots V+XI
Number of steers	12	12	12
	Growth data		
Initial weight, lb.	512	514	512
Final weight, lb.	1014	1090	1093
Total gain, lb.	502	576	581
Daily gain, lb.	2.25	2.58*	2.61*
Increase in daily gain, %		14.7	16.0
	Daily feed consumption		
Ground ear corn, lb.	10.40	11.12	11.42
Supplement A, lb.	2.30	2.30	2.30
Roughage, lb.	5.43	5.65	5.75
Minerals, lb.	.05	.08	.08
Increase in daily feed, %		5.2	7.4
	Feed per 100 lb. gain		
Ground ear corn, lb	462	430	438
Supplement A, lb.	102	89	88
Roughage, lb.	241	218	220
Minerals, lb.	4	3	3
Feed saved, %		8.5	7.4
	Carcass data		
Dressing percentage	60.0	58.9	59.8
Carcass grades			
Top choice	2	1	1
Av. choice	6	2	2
Low choice	1	5	5
Top good	3	2	3
Av. good	0	0	1
Low good	0	0	0

* Gained significantly more than Lots I and VII.

diethylstilbestrol per day for 233 days actually consumed 2330 mg. or more than 64 times the dosage which was implanted.

The Effect of an Antibiotic. The feeding of 80 mg. chlortetracycline per steer daily resulted in an average of 5.4% increased rate of gain (table 4). These differences were not statistically significant, but did indicate a trend.

Carcass Data. The feeding of chlortetracycline resulted in apparently

TABLE 4. EFFECT OF CHLORTETRACYCLINE ON THE GAIN OF STEERS FED OR IMPLANTED WITH DIETHYLSTILBESTROL

Level of DES	Daily gain, lb.		
	No antibiotic	80 mg. CTC per day	% increase
No hormone	2.19	2.31	5.5
10 mg./day, orally	2.52	2.65	5.2
12 mg. implant	2.31	2.59	12.1
24 mg. implant	2.36	2.47	4.7
36 mg. implant	2.59	2.63	1.5
48 mg. implant	2.47	2.61	5.7
Average	2.41	2.54	5.4

improved carcass quality (table 5). Whereas 72% of the carcasses from chlortetracycline-fed cattle graded U. S. Choice, only 47% of the carcasses from cattle which had not receive chlortetracycline graded U. S. Choice.

Side Effects. During the 233-day experimental period mammary development, particularly an increase in teat length, was apparent in all lots of estrogen treated cattle, whether by implantation or oral administration. In the implanted lots this effect was most noticeable in the first two months, and was more apparent in the cattle treated with 36 or 48 mg. of diethylstilbestrol. Toward the end of the trial there was a gradual regression of mammary changes in the implanted cattle. These changes caused no difficulty in any of the animals at any time.

Discussion

The method of subcutaneous implantation of diethylstilbestrol has proven equally as effective as the *per os* method. In this research, steers implanted with 36 mg. of diethylstilbestrol gained at the rate of 2.61 lb. per day,

TABLE 5. THE EFFECT OF CHLORTETRACYCLINE ON CARCASS QUALITY

	Top choice	Average choice	Low choice	Top good	Average good	Low good
	(percent of carcasses falling in each grade)					
Lots I through VI, no CTC	8	17	22	47	3	3
Lots VII through XII 80 mg. CTC	3	36	33	28	0	0
	(number of carcasses falling in each grade)					
Treatment						
No CTC	2	5	7	14	1	1
80 mg. CTC/day	0	8	13	9	0	0

whereas those fed 10 mg. per day gained 2.58 lb. per day over a 233-day fattening period. The control animals gained 2.25 lb. per day. In a 98-day feeding trial Aunan *et al.* (1956), reported daily gains of 2.25 lb., 2.74 lb., and 2.68 lb., respectively, for control steers, for those implanted with 36 mg. and for those fed 10 mg. of diethylstilbestrol per day. The Kansas Station (Good *et al.*, 1957) obtained data which indicated increased gains by the implantation of 84 mg. over the feeding of 10 mg. diethylstilbestrol. Daily gains were as follows: control, 2.71 lb., 10 mg. diethylstilbestrol fed per steer per day, 2.97 lb.; implanted with 84 mg., 3.28 lb. The data from three trials reported by Klosterman (1956) indicated daily gains of 1.97 lb. for control steers; 2.22 lb. for 36 mg. implanted steers; and 2.31 lb. for steers fed 10 mg. per day. However, in a pasture trial the Purdue Station (Beeson *et al.*, 1957) reported a slight growth depression for steers fed diethylstilbestrol (control lot gained 2.71 lb. per day; those fed 10 mg. per day gained 2.56 lb. per day), whereas an increase in gain was reported for cattle implanted with either 24 mg. (3.07 lb. per day), or 36 mg. (3.19 lb. per day).

Side effects such as elevated tail heads and lowered loins were observable, and of approximately the same magnitude, in both the orally treated and the subcutaneously implanted lots. Good *et al.*, (1957) reported side effects from both methods of administration, as indicated by increased teat length, increased size of the sex organs, increased diameter and length of the penis, and hypertrophy of the bulbo-urethra and of the prostate glands. Aunan *et al.*, (1956) reported side effects from both methods of administering diethylstilbestrol, but more severe effects were noted in the implanted group.

Summary

The administration of diethylstilbestrol either in the feed or by implantation of 36 mg. under the hide resulted in increased gains of from 14.7 to 16.0%. Both methods of administration apparently exert about the same growth stimulatory effect in drylot. Both methods of administration of diethylstilbestrol resulted in from 9 to 10% increase in the consumption of ground ear corn, but the increase in gain was sufficient that the diethylstilbestrol-treated cattle required from 7.4 to 8.5% less total feed per unit of gain than cattle which had not been treated with diethylstilbestrol.

The feeding of 80 mg. chlortetracycline per day resulted in a 5.4% increase in gain over lots that received no chlortetracycline.

The feeding of 80 mg. chlortetracycline resulted in upgrading of the carcasses. Whereas 72% of the carcasses from chlortetracycline-fed cattle graded U. S. Choice, only 47% of the carcasses from cattle which had not received chlortetracycline graded U. S. Choice.

Side effects were the same regardless of whether steers were implanted with 36 mg. of diethylstilbestrol or fed 10 mg. diethylstilbestrol per head daily.

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FURTHER STUDIES CONCERNING THE EFFECTS OF FATS IN SHEEP RATIONS

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Ward *et al.* (1957) reported that the addition of 3% or more of corn oil to ruminant rations containing high levels of corn cobs or cottonseed hulls caused significant reductions in the digestibilities of most ration components, especially crude fiber, and in weight gains. The detrimental effect of corn oil was completely counteracted by alfalfa ash if the level of corn oil did not exceed 5% of the total ration and only partially when the level of corn oil was higher. The work reported herein was designed for the purpose of studying (1) the physiological and mechanical effects of high levels of fats in sheep rations and (2) the effect of alfalfa ash upon intra-ruminal pH in the sheep.

Experimental Procedure

Trial 1, Growth. Forty-five western-type ewe lambs weighing 59 ± 12 lb. were divided on the basis of weight into five approximately equal groups. These groups were than randomly assigned to the designated lots and treatments. A 12- to 14-hour shrink period (Ward *et al.*, 1957) preceded the initial and final weights. During the 28-day trial, the sheep had free access to the designated rations and water.

The percentage composition of the rations is shown in table 1. The calculated crude protein content of the control ration (ration 1) was 10% while that of the other rations was increased in order to obtain a calculated total digestable nutrient to crude protein ratio of 6.5:1. Mineral salts were added in amounts calculated to be equivalent to the amount of alkali required to saponify the added animal fat. The saponification number of the fat was 189, which is similar to tri-stearin.

The alfalfa ash was prepared by the method of Tillman *et al.* (1954). Statistical analysis of the data was by the analysis of variance method of Snedecor, (1946).

Trial 2, Digestion. It was found in the previous trial that a high level of animal fat caused a depression in gains; which is in conformance with the earlier work of Ward *et al.* (1957). The purpose of the present trial was to ascertain whether the animal fat used in trial 1 affected the digestibilities of dry and organic matter.

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TABLE 1. PERCENTAGE COMPOSITION OF RATIONS AND RESULTS OF THE GROWTH AND DIGESTION TRIALS

	Trial 1					Trial 2		Trial 3		
Ration number	1	2	3	4	5	1	2	1	2	3
Items:										
Cottonseed hulls	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Ground milo	58.5	31,2	26.4	24.8	23.6	45.0	15.4	45.0	25.4	25.4
Cottonseed meal	4.9	17.2	18.4	18.9	19.2	18.4	33.0	18.4	28.0	28.0
Calcium carbonate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate [a]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Quadrex [b]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Animal fat		15.0	15.0	15.0	15.0		15.0			
Alfalfa ash			3.6							
$NaHCO_3$				4.7						
$KHCO_3$					5.6					
Corn oil									10.0	10.0
Av. initial weight, lb.	59	59	59	59	59			57	55	58
Av. daily feed, lb.	2.4	1.5	1.7	1.3	1.0			2.00	1.73	1.71
Av. daily gain, lb.	0.17	—0.04**	—0.04**	—0.12**	—0.15**			0.28	0.20*	0.19*
	(.01) [c]	(.02)	(.01)	(.03)	(.04)			(.01)	(.04)	(.02)
No. animals started	9	9	9	9	9	5	8	4	4	4
No. animals died	0	1	1	2	3	0	0	0	0	0
Feed/lb. gain, lb.	13.4							7.1	8.8	9.1
Dry matter digestibility, %						63.8	45.1*			
						(0.9)	(5.6)			
Organic matter digestibility, %						65.7	49.2*			
						(0.5)	(4.8)			

[a] Feeding grade dicalcium phopshate.
[b] Vitamins A and D supplement courtesy of Dr. M. Hochberg, NOPCO Chemical Co.
[c] Numbers below are standard deviations of the mean.
* Different from basal at the 5% probability level.
** Different from basal at the 1% probability level—see A.O.V. data (table 2) for further comparisons.

Thirteen western-type wether lambs weighing 78 ± 16 lb. each were divided on the basis of weight into two groups, one group of five receiving the basal ration (ration 1) while the others received the fat-containing ration (ration 2). Daily allowances of rations 1 and 2 were 545 and 454 grams, respectively; these amounts being approximately isocaloric and iso-nitrogenous.

The animals were fed one-half of their rations twice daily in stanchions as described by Tillman *et al.* (1954). Water was available except when the animals were eating. A 10-day preliminary period preceded a 7-day collection period during which the feces were collected by the method of Garrigus and Rusk (1939). Fecal collections were made once daily and the total collection was dried in a forced-draft oven. Other details of this procedure have been described by Tillman *et al.* (1954). Chemical analyses of feed and feces were by accepted methods of the A.O.A.C. Other details were as described in trial 1.

Trial 3, Growth. The results of the two previous experiments confirms the idea that in sheep high levels of either animal or vegetable fats depresses both gains and the digestibility of most ration components. Ward *et al.* (1957) advanced the idea that fat could be coating the roughage portion, thereby preventing the breakdown of cellulose and other complex carbohydrates by rumen microorganisms. The present study was designed to test that hypothesis.

Fifteen western-type lambs weighing 57 ± 12 lb. were divided on the basis of weight into three equal groups of five per group. These were kept in individual pens that measured 3 x 6 feet during the 42-day test. A 12-hour shrink period preceded the initial and final weights. Each lamb receiving the control ration (ration 1) had a daily allowance of 821 gm. while those receiving the fat-containing rations had a daily allowance of 680 gm.; these amounts being approximately isocaloric and iso-nitrogenous.

The compositions of the rations are shown in table 1. The control ration (ration 1) is the same one used for that purpose in trial 2. The fat-containing rations (rations 2 and 3) had the same composition but differed in the way they were fed. In ration 2, the corn oil was mixed with the other ingredients of the ration and the animal allowed to consume it from one container. In ration 3, the corn oil was mixed with the concentrate portion of the ration only and fed from one container while the roughage portion of the ration was offered at the same time but in another pail. Other details were as described in trial 1.

Trial 4, Blood and Urine Ketone Bodies. It seems that the primary effect of high levels of fat in ruminent rations is to depress digestibility. Depression of appetite is also noted when the fat is added. As inappetence is also associated with ketosis in sheep, this trial was initiated for the purpose of comparing the levels of blood and urine ketone bodies of

lambs fed high fat diets with those of lambs fed predominately carbohydrate diets.

Six western-type wether lambs weighing 70 ± 5 lb. each were placed in individual pens (trial 3) and given 780 gm. of the basal ration (ration 1, trial 3) for 10 days. They were then transferred to metabolism units (Briggs and Gallup, 1949) and continued on feed for another 2 days while they became accustomed to the units. Urine from each animal was then collected during a 24-hour period. At the end of the collection period, 4 of the above animals were given a daily allowance of 685 gm. of a 10% corn oil ration (ration 2, trial 3) for 10 days, while 2 animals were continued as before. Urine was then collected from each animal during a 24-hour period. Blood samples by jugular puncture were taken at the end of the collection period.

The 4 lambs which had previously received the 10% fat ration were then given 680 gm. of a 15% fat ration (ration 2, trial 2) for 10 days, while 2 animals were kept on the basal ration as before. At the end of the standardization period blood and urine samples were also obtained as before.

After sugar and other interfering substances were removed by the copper sulfate-calcium hydroxide procedure of Van Slyke (1917), the ketone body content of urine and blood was determined by the salicylaldehyde method of Behre (1940). The results are expressed as mg. of acetone per 100 ml. blood or urine. Other details of experimental procedure were described in trial 2.

Trial 5, Intra-Ruminal pH. Three months before the start of the experiment, three western-type wether sheep were equipped with rumen fistula plugs.[4] This plug, which was a modification of that one described by Hentshl *et al.* (1954), had an orifice two inches in diameter and was permanantly inserted. Foam rubber gaskets placed both internally and externally were effective in preventing leakage around the fistulas.

The pH measurements were made using a locally constructed pH meter[5] which permitted the use of self-made electrodes of appropriate design for these studies. These electrodes were of sufficient length that the electrical lead wires from the electrodes to the pH meter did not at any time enter the rumen cavity. For the readings, the electrode tops were immersed about two inches below the surface of the liquid in about the midsection of the ventral sac of the rumen. Several readings were taken in this area to determine repeatability, thus, each reported value represents the average of several readings.

The animals were kept in individual pens and fed once daily. When not eating, they were allowed the freedom of the pen and free access to water. During the first phase of the trial all animals received a daily

[4] Dr. E. W. Jones, Department of Veterinary Surgery, performed the surgery necessary for insertion of the fistula plugs.

[5] Similar to a type first designed by Dr. M. J. Johnson, Biochemistry Department, University of Wisconsin, Madison.

allowance of 550 gm. of a basal ration (ration 1, trial 3). Readings of intra-ruminal pH were made at various times after feeding for 8 days. The pH value reported for each given time after feeding (figure 1) represents an average of several readings made on different days; this average being obtained by converting the individual values to their

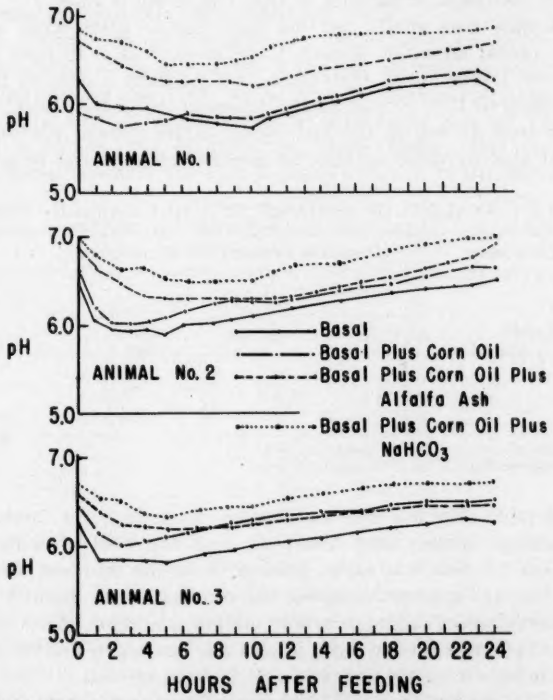

Figure 1. The effect of various treatments upon the intra-ruminal pH values of sheep.

respective antilogarithms and these averaged. The mean was then reconverted to its equivalent pH value.

When the first phase was completed, the animals were each given the basal diet plus a daily allowance of 55 gm. of corn oil. Intra-ruminal pH values were taken as before. Observations on the effect of this treatment upon the intra-ruminal pH were made for 4 days. At the end of this phase, 45 gm. of alfalfa ash were added to the fat-containing ration and the pH measurement taken over a 9-day period. At the end of this phase, the alfalfa ash was replaced by 25 gm. of $NaHCO_3$ and the pH measurements taken during a 4-day period.

Results and Discussion

Trials 1, 2 and 3. The results of these trials are shown in table 1. The high level of animal fat caused a drastic reduction in weight gains. None of the mineral additives improved gains when added to the high-fat rations. In fact, the added bicarbonates appeared to depress gains further. Statistical treatment of the data of trial 1 is shown in table 2.

The finding that alfalfa ash did not improve gains when added to the high animal fat ration appears to be at variance with those of Ward *et al.* (1957), who found that alfalfa ash partially alleviated the detrimental effects of 10% corn oil in the rations of cattle and sheep. However, since the level as well as the kind of fat in the present studies differed from that used by those workers, no direct comparison can be made.

TABLE 2. ANALYSIS OF VARIANCE OF DAILY GAINS IN TRIAL 1

Source of variance	Degrees of freedom	Mean square	F value
Total	37		
Treatment	4	224.1	7.7**
1 vs. 2, 3 and 4	1	782.0	26.9**
2 and 3 vs. 4 and 5	1	110.7	3.8[a]
2 vs. 3	1	0.3	0.01
4 vs. 5	1	3.4	0.12
Error	33	29.1	

** Difference significant at the 1% probability level.
[a] Difference significant at the 10% probability level.

Considerable difficulty was experienced in starting the lambs on the fat-containing rations. After about 20 days the wool of some animals on the high fat diets was easily detached from the skin and wool-pulling in these lots was common. Scouring was common to the animals receiving the unsupplemented high-fat ration (ration 2); while hard and gray-colored pellets were formed when alfalfa ash was added (ration 3). Seven lambs were lost during the trial, all on the high-fat rations.

In trial 2, the addition of 15% animal fat caused decreases ($P>.05$) in the digestibilities of both dry and organic matter. This is an agreement with the findings of Ward *et al.* (1957) when they fed corn oil. The addition of corn oil to the basal ration of trial 3 decreased gains and the efficiency of feed utilization; however, the method of feeding had no influence upon this effect.

Trial 4. The results of this trial are shown in table 3. Neither the 10 nor the 15% level of corn oil caused an increase in the level of blood or urine ketone bodies; indicating that these sheep did not have ketosis. The work of Sampson and Boley (1940) bear on this point. They found that 75% of the blood collected from 60 pregnant ewes contained 3 mg.% or less of ketone bodies (expressed as acetone). Levels of 7 to 10 mg.% indicated an early stage of ketosis while in advanced stages higher levels

were observed. In the case of urinary ketone bodies, Sampson and Hayden (1936) found that healthy sheep had levels from 0.5 to 5.0 mg.%, while in animals having ketosis the level ranged from 15 to 100 mg.%.

One of the sheep receiving the basal diet declined to eat or drink during a 4-day period. The blood obtained from him before he died on the 4th day contained 11.1 mg.% acetone. Unfortunately the animal failed to urinate during this self-inflicted fast period.

Trial 5. Figure 1 shows diurnal curves derived from composite ruminal pH values taken at various times after the animals were fed. These curves illustrate the effect of the various treatments upon the diurnal variation in ruminal pH values. When the basal ration was fed, all animals produced a similar type of curve, i.e., after feeding the ruminal pH values dropped reaching the lowest point within 4 to 10 hours. After-

TABLE 3. EFFECT OF ADDED FAT UPON BLOOD AND URINE KETONE BODIES

Treatment	Number of animals	Urinary ketones		Blood ketones
		mg./100 ml.[a]	Total	
			mg./day	mg./100 ml.
Basal	9	3.6	30.0	4.8[b]
10% corn oil	4	2.7	38.6	3.0
15% corn oil	4	2.4	13.3	4.8

[a] Expressed as acetone.
[b] Average of 3 animals.

wards, the pH values began to rise and reached the highest values approximately 20–24 hours after being fed which is in conformance with the results of Nottle (1956). The ruminal diurnal pH values reported herein apparently varied inversely with the ruminal diurnal fatty acid concentration values of sheep reported by McClymont (1951).

No changes in ruminal pH values were noted when corn oil was added to the basal ration. Approximately 4 to 6 hours after the corn oil ration was fed, however, the rumen became filled with a frothy foam within which feed particles were suspended. When the fistula cap was removed, there was sufficient pressure to push large quantities of the foam out of the animal. This foam was not broken up in *in vitro* studies by the silicone anti-foaming agents, dilute sodium hydroxide or sodium bicarbonate. In fact, the sodium bicarbonate seemed to increase the amount of foam. The animals receiving corn oil plus alfalfa ash or sodium bicarbonate drank more water and apparently had less ruminal foam than did those receiving only the fat-containing ration.

The addition of alfalfa ash or sodium bicarbonate apparently reduced the hydrogen-ion concentration with resultant higher pH values throughout the day. In each animal, the feeding of sodium bicarbonate gave

higher values than alfalfa ash. A relationship between the pH of the rumen and the absorption of fatty acids from it was shown by Pfander and Phillipson (1953). They found that by lowering the ruminal pH from 6.4 to 5.0 the absorption of volatile fatty acids was increased from 21 to 98 milli-equivalents per hour. Gray (1948) reported there was no absorption of acetic acid or sodium acetate when the ruminal pH was 7.5 or higher. The work of Ward *et al.* (1957) showed conclusively that 10% corn oil reduced gains and digestibility of ration components by sheep and cattle and that the addition of alfalfa ash partially alleviated the depressing effect of corn oil. Certainly the effect of alfalfa ash upon diurnal ruminal pH values does not offer an explanation for its beneficial effects. When sodium or potassium bicarbonate was fed in trial 1, the animals, within one day, refused to eat and 5 of 18 lambs died during the 28-day test. The gastrointestinal tract of these animals were devoid of food, while the other animals in these groups ate only very small quantities of their diets. On the second day of trial 5, all of the sheep refused the ration containing sodium bicarbonate and these animals were given their daily allowances of the ration through the fistula.

Because of the number of variables affecting ruminal pH values, combined with the limited number of animals alloted each treatment, the authors are not prepared to discuss the nutritional implications of these findings. It is possible that the shift in ruminal pH toward the alkaline side in case of sodium bicarbonate could possibly affect ruminal micro-organisms, suggesting that to maintain a favorable rumen environment for the digestion and absorption of nutrients, it is essential that the pH of the ingesta be maintained within the optimum range. Certainly more studies are necessary for an evaluation of these factors.

Summary

In growth and digestibility studies with sheep, it was found that the inclusion of 15% animal fat in a basal ration containing cottonseed hulls significantly reduced the digestibilities of dry matter and organic matter, and the weight gains. The addition of alfalfa ash, sodium bicarbonate, or potassium bicarbonate to the high fat ration did not improve weight gains; in fact, both bicarbonates tended to depress appetites and gains. In another experiment, it was shown that the addition of 10% corn oil reduced gains regardless of whether it was absorbed by the concentrate or roughage portion of the ration.

Neither 10 nor 15% corn oil in the rations of sheep increased the accumulation of blood and urine ketone bodies. The addition of corn oil did not seem to affect diurnal ruminal pH values of sheep, whereas alfalfa ash and sodium bicarbonate tended to reduce the hydrogen-ion concentration. The addition of corn oil caused an accumulation of a frothy foam in the rumen. Some nutritional implications of these observations were discussed.

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MOLYBDENUM AS A DIETARY ESSENTIAL FOR LAMBS [1, 2]

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THE presence of a mineral, or a combination of minerals, in alfalfa ash which stimulates weight gains of ruminants fed poor quality roughages has been reported by numerous investigators (Burroughs *et al.*, 1950; Swift *et al.*, 1951; Tillman *et al.*, 1954; Chappel *et al.*, 1955; Rhodes *et al.*, 1956). Brooks (1954) reported a rather complete spectrographic analysis of three alfalfa ashes, each of which produced a different weight gain response when fed to lambs. The ash which promoted the greatest response was characteristically higher in molybdenum and lower in copper than the other two.

Molybdenum has been recognized as a dietary essential for bacteria (Bortels, 1930) and higher plants (Arnon and Stout, 1939). Teresi *et al.* (1942) concluded that any requirement of the growing rat for this mineral was met by 0.5 mcg. per day. The toxic role of molybdenum in ruminant nutrition has received extensive study and review (Dick, 1956; Underwood, 1956) since the original observations of Ferguson *et al.* (1938).

The essential dietary nature of molybdenum for the rat was first implied by the studies of de Renzo *et al.* (1953) who showed it to be the cofactor for intestinal and liver xanthine oxidase. Subsequent work by these workers and others (O'Dell *et al.*, 1955; Higgins *et al.*, 1956) has not indicated any effect of low molybdenum intake upon growth or the purine metabolism of the rat. A slight reduction in growth rate of chicks fed low molybdenum rations has been reported when tungstate was used as a molybdenum inhibitor (Higgins *et al.*, 1956). Reid *et al.* (1956) reported reduced weight gains in chicks and poults fed rations containing an unspecified amount of molybdenum as compared to rations with added molybdenum.

This paper summarizes experiments, conducted over the past three years, which collectively demonstrate a beneficial role of molybdenum in ovine nutrition. A preliminary account of the initial results of this work has been reported elsewhere (Pfander *et al.*, 1955).

Experimental

In Vitro Incubation. The *in vitro* technique used was that described by Brooks *et al.* (1954). Donor wethers, previously fitted with permanent fistula (Phillipson and Innes, 1939), were maintained on alfalfa hay.

[1] Journal Series Paper No. 1730 approved by the Director of the Missouri Agricultural Experiment Station.
[2] Supported in part by a grant from Swift and Company, Chicago, Illinois. Dr. George B. Garner assisted with the *in vitro* tests.

Rumen ingesta, removed by vacuum and filtered through four layers of cheesecloth, was used as the inoculum.

Additions of alfalfa ash, molybdenum, and molybdenum in combination with copper were made to the incubation tubes. The amounts added per tube were: (1) alfalfa ash, 10 mg. (2) copper, 0.8 mcg. and molybdenum, 0.27 mcg. (3) molybdenum, 0.27 mcg. and (4) molybdenum, 2.2 mcg. Since each incubation tube contained 0.5 gm. dry matter, these additions represented (1) 2% of alfalfa ash (2) 1.6 p.p.m. copper and 0.54 p.p.m. molybdenum (3) 0.54 p.p.m. molybdenum and (4) 4.4 p.p.m. molybdenum on the dry matter basis. Cellulose digestibility was calculated as the percentage of cellulose which disappeared during the incubation period of 44 hours.

Dried Rumen Bacteria. A composite sample of rumen ingesta was collected and filtered as described above. The filtered liquor was then centrifuged at 1,700 r.p.m. for 5 minutes to obtain a supernatant which appeared to be free of protozoa and ration residue when examined under a microscope. This supernatant was fed through a Sharples Ultracentrifuge at a rate of 4 liters per hour and a bowl speed of 35,000 r.p.m.; the bacteria removed from the bowl were washed three times in 95% ethanol and twice in anhydrous ether. Following the final ether washing, the sample was ashed at 580° C. Iron, copper, zinc, cobalt and molybdenum were estimated with a 480 cm. adjustable grating spectrograph (Jarrell-Ash Co.) after suitable chemical separations. Molybdenum was estimated by visual inspection of the line at 3170A°.

Feeding Trials, 1955 and 1956. Semi-purified rations containing mineral mixtures with and without added molybdenum were used in feeding trials with wether lambs. Rations were processed (Ellis *et al.*, 1956) to make them more palatable. The ingredients of these rations and the chemical analysis of the ration used in 1956 are shown in table 1.

Six lambs were fed in 1955 and 12 in 1956. The lambs were fed in individual wooden feeding stalls having galvanized tin feeders during the feeding trials. Except at feeding time, they were together in a graveled lot. All lambs were fed 800 gm. ration per day during the 1955 experiment. Feed consumption was quite variable initally among the lambs in the 1956 feeding trial, therefore, these lambs were paired on the basis of body weight and past feed consumption. One lamb in each pair received the basal ration and the other lamb the molybdenum supplemented ration. Intakes of pairs varied from 600 to 900 gm. per day for the first 21 days, were constant at 900 gm. per day from the 21st. to the 35th. day, and were constant at 1 kg. per day for the remainder of the test.

Digestion Trial, 1956. Six lambs, three receiving the molybdenum supplemented ration and three receiving the basal ration, were placed in metabolism stalls during the last 10 days of the 1956 feeding trial. The feces collected on the last 6 days were saved for analysis and the digestion coefficients calculated for this period. Immediately following the 6-day

collection period, the lambs which had received the molybdenum supplemented ration were changed to the basal ration and those which had received the basal ration were changed to the molybdenum supplemented ration. A 6-day fecal collection period was initiated 14 days following this ration change.

Analytical. Feed and fecal samples were analyzed for cellulose by the method of Crampton and Maynard (1938). The remaining proximate constituents and sulfur were determined by procedures of the A.O.A.C. (1945) except that N.F.E. was determined as 100—[ether extract + cellulose + water + ash + (N x 6.25)]. Ration copper and molybdenum content were determined spectrographically after preliminary chemical concentration by the methods of Mitchell (1948).

TABLE 1. COMPOSITION AND ANALYSIS OF SEMI-PURIFIED RATIONS[a]

Ingredient		Analysis		
	%		Molybdenum supplemented	Basal
Purified roughage[b]	19.3	Water	6.9%	7.0%
Beet pulp, plain	21.7	Ether extract	2.1%	2.0%
Starch	26.9	Ash	5.2%	5.2%
Cerelose	14.7	Nitrogen	1.72%	1.71%
Minerals[c]	4.9	Cellulose	22.4%	22.3%
Lard	2.0	Sulfur	0.31%	0.30%
Casein	10.3	Copper (p.p.m.)	13.41	10.49
Vitamin supplement[d]	0.2	Molybdenum (p.p.m.)	2.36	0.36
	100.0			

[a] Analysis for semi-purified ration used during summer 1956 feeding and digestion trial.
[b] Solka floc, the Brown Company, Berlin, New Hampshire.
[c] Mineral mixture of Ellis *et al.* (1956), compounded from either reagent or purified grade minerals. $Na_2MoO_4 \cdot 2H_2O$ omitted from mineral mixture used for basal ration.
[d] Stabilized vitamin A and D guaranteed to contain 2,250 I.U. vitamin A and 400 I.U. vitamin D per gram.

Results

In Vitro Incubation. The results of three separate incubation trials are summarized in table 2.

Cellulose digestion was increased equally by either the alfalfa ash or the copper-molybdenum additions in each of the three trials. When the three trials were combined and analyzed by analysis of variance, increases due to addition of alfalfa ash or the copper-molybdenum combination approached significance ($P<0.1$). Additions of molybdenum alone at low levels (0.27 mcg.) did not significantly increase cellulose disappear-

ance. Addition of higher levels of molybdenum (2.2 mcg., trial 2) seemed to slightly depress cellulose digestion. Brooks (1954) has reported that additions of 10 mcg. molybdenum alone to similar "artificial rumens" significantly depressed cellulose digestion. These results suggest that copper and molybdenum in combination may account for the activity of alfalfa ash in stimulating cellulose digestion *in vitro*. It should be noted that the crude inoculums used here probably contained appreciable amounts of copper and molybdenum, which may account for the small responses obtained. (See analysis of bacteria reported below)

TABLE 2. THE EFFECT OF ALFALFA ASH, MOLYBDENUM-COPPER COMBINATION, AND MOLYBDENUM ON *IN VITRO* CELLULOSE DIGESTION

Trial	Tubes per treatment	Treatments				
		Basal	Basal+ 10 mg. alfalfa ash	Basal+ .27 mcg. Mo .8 mcg. Cu	Basal+ .27 mcg. Mo	Basal+ 2.2 mcg. Mo
		%	%	%	%	%
1	6	27.1±9.7[a]	31.5±4.9	30.5±6.5	27.1±5.4	.....
2	4	7.4±5.7	16.9±2.5	16.1±5.9	14.0±3.7	6.3±2.7
3	6	59.8±5.2	60.5±4.3	61.4±2.8	57.1±4.3	.....
		34.4	38.7	38.4	35.3	

[a] Percentage of total cellulose which disappeared and standard deviation.

Dried Rumen Bacteria. The dried bacteria contained 9.41% ash. The ash contained, in approximate p.p.m.: iron, 300; zinc, 200; copper, 100; cobalt, trace; and molybdenum, 10. Thus, the dried, fat free cells contained about 1 p.p.m. molybdenum. Admittedly, some of this molybdenum, as well as some of the other trace minerals, may have been derived from the metal centrifuge bowl. No satisfactory control was devised to correct for this.

Feeding Trial, 1955. The results of this experiment are summarized in table 3.

Lambs receiving the semi-synthetic ration with supplemental molybdenum made larger gains ($P<0.01$) than those on rations without added molybdenum. If lamb 31 is not considered a "normal" individual due to its poor weight gain and is excluded from consideration, the difference due to ration is still significant ($P<0.05$).

Feeding Trial, 1956. The growth curves for the lambs receiving the basal and molybdenum supplemented rations in 1956 are shown in figure 1.

Differences in weight gains favoring the molybdenum supplemented lambs were significant ($P<0.01$) 21 days after the start of the experiment. The group differences remained significant ($P<0.01$) for the re-

TABLE 3. WEIGHT GAINS OF LAMBS FED SEMI-PURIFIED RATIONS WITH AND WITHOUT ADDED MOLYBDENUM (SUMMER 1955)

Lamb number	Ration	Initial weight	Final weight	Weight gain (70 days)	Average daily gain (70 days)
		lb.	lb.	lb.	lb.
31	Basal	66	65	−1	0
36	Basal	78	90	12	0.17
33	Basal	80	92	12	0.17
Mean		74.7	82.3	7.6	0.11
37	Basal+Mo	70	91	21	0.30
29	Basal+Mo	74	93	19	0.27
28	Basal+Mo	61	78	17	0.24
Mean		68.3	87.3	19.0	0.27

mainder of the experiment. The growth curves shown in figure 1 indicate that approximately 14 days were required before the favorable effects of molybdenum were manifested. Differences in body weight between the two groups widened more rapidly when ration allowances were increased or when the lambs were in metabolism stalls and, therefore, less active.

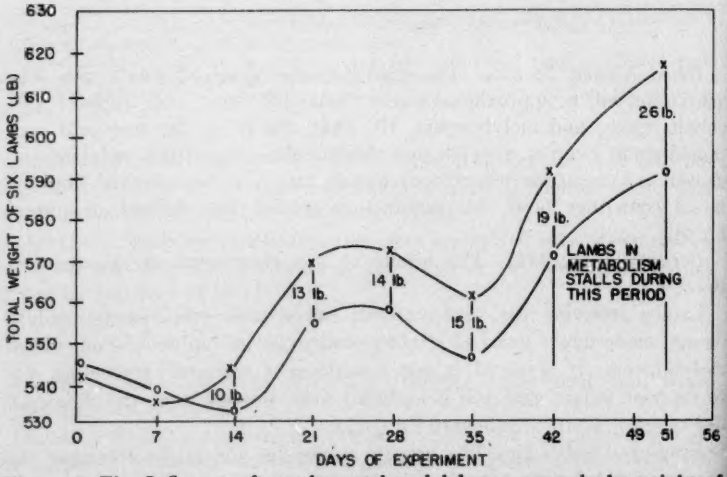

Figure 1. The Influence of supplemental molybdenum upon body weight of six lambs.

Legend:

×———× 0.3 gm. $Na_2MoO_4 \cdot 2H_2O$ per 100 lb. ration.

O———O no added $Na_2MoO_4 \cdot 2H_2O$.

The average daily gain during the 51-day period was 0.15 lb. for lambs receiving the basal ration and 0.23 lb. for lambs receiving the molybdenum supplemented ration.

Digestion Trial, 1956. The results pertaining to cellulose digestion are summarized in table 4.

The digestibility of cellulose in the molybdenum supplemented ration was greater ($P<0.01$) than in the basal ration. Only one lamb failed to digest more cellulose when receiving supplemental molybdenum. The large variation in cellulose digestibility among animals appears to be

TABLE 4. THE DIGESTIBILITY OF CELLULOSE AFTER 45–51 DAYS ON A CONSTANT RATION AND 14–20 DAYS FOLLOWING EITHER THE REMOVAL OR ADDITION OF SUPPLEMENTAL MOLYBDENUM

Lamb number	Supplemental Mo		Digestibility[a]		
	Initial ration	Final ration	Initial ration	Final ration	Difference (+MO)−(Basal)
	days 45–51	days 51–71	%	%	
41	+[b]	−[c]	35.0	36.3	−1.3
45	+	−	46.2	40.5	+5.7
51	+	−	52.6	40.7	+11.9
Mean difference (+Mo)−(Basal)					+5.4
48	−	+	28.4	36.9	+8.5
47	−	+	19.8	31.5	+11.7
49	−	+	31.4	40.7	+9.3
Mean difference (+Mo)−(Basal)					+9.8

[a] Digestibility based on total fecal collections taken from the 14th through the 20th day after changing from initial ration.

[b] Indicates 300 mg. sodium molybdate dihydrate was added per 100 lb. ration.

[c] Indicates no supplemental molybdenum.

characteristic for semi-purified rations. Molybdenum seemed to have little residual effect since the removal of added molybdenum resulted in decreased cellulose digestibility in 14 days.

The digestibility of ration constituents other than cellulose are summarized in table 5. Supplemental molybdenum increased, non-significantly, the digestibility of nitrogen and organic matter. The only reversal occured with lamb 45 who possessed higher coefficients of digestibility for each of these ration constituents when receiving the basal ration. The digestibility of nitrogen was altered more by supplemental molybdenum than was organic matter digestibility.

Discussion

The results of the *in vitro* experiment and the digestion trial suggest that molybdenum stimulates lamb weight gains by increasing cellulose degradation by rumen microorganisms. Although the *in vitro* results do

not show statistically significant differences favoring molybdenum additions, the similarity of this trend with the digestion trial results is of significance. The effect of molybdenum on *in vivo* ration digestibility reported here is similar to the effect of alfalfa ash on ration digestibility reported by Swift *et al.* (1951). These investigators found that alfalfa ash additions to high corn cob rations significantly increased the digesttibility of crude fiber but did not significantly increase the digestibility of other ration constituents. Burroughs *et al.* (1950); Tillman *et al.* (1954); Chappel *et al.* (1955); Summers *et al.* (1956) and others have shown a similar effect of alfalfa ash upon crude fiber digestibility.

TABLE 5. THE EFFECT OF SUPPLEMENTAL MOLYBDENUM UPON THE APPARENT DIGESTIBILITY OF RATION CONSTITUENTS

	Nitrogen		N.F.E.		Ether extract		Organic matter	
Lamb number	Mo supplemented	Basal	Mo supplemented	Basal	Mo supplemented	Basal	Mo supplemented	Basal
47[a]	64.9	62.6	89.2	88.3	82.5	85.3	69.2	67.8
48	69.3	67.0	90.5	90.1	85.7	86.3	71.9	71.6
49	71.7	67.7	90.8	90.0	86.7	84.1	73.9	72.3
41	69.5	66.8	92.9	90.3	86.8	85.1	74.0	73.9
45	59.1	66.8	88.7	91.9	83.8	87.0	74.2	75.4
51	68.3	64.5	90.5	90.0	85.9	85.1	78.1	74.1
Mean	67.1	65.9	90.4	90.1	85.2	85.5	73.6	72.5

[a] The sequence with which lambs received the basal and molybdenum supplemented rations is the same as indicated in table 4.

The growth curves of lambs during the 1956 trial (figure 1) indicated that the beneficial effect of molybdenum occurs as early as 7 to 14 days following its addition to the ration. The demonstrated increase in cellulose digestibility 14 days following addition of supplemental molybdenum is further indicative of a rapid response to molybdenum. Brooks *et al.* (1954) reported a similar rapidly occurring beneficial effect of alfalfa ash 14 days following its addition to a cottonseed hull ration.

A specific function of molybdenum in stimulating lamb weight gain, exclusive of the effect on cellulose digestibility as mediated through the rumen microorganisms, has not been resolved. Three pairs of sheep have been kept on the same rations for 7 months. No difference in the general appearance, health, or wool production has been observed between the molybdenum supplemented and non-supplemented lambs. No growth promoting effect of molybdenum could be found in the literature for any species except the chick and poult (Higgins *et al.*, 1956; Reid *et al.*, 1956). Molybdenum supplementation has produced a growth response in these species only when a molybdenum inhibitor was used (Higgins *et al.*,

1956) or chicks and poults from dams on low molybdenum rations were used (Reid *et al.*, 1956). Addition of extremely small amounts of molybdenum produced a growth response under these conditions. Contrasted to experiments with chicks and poults, an expression of molybdenum deficiency was easily demonstrated to occur in the lambs reported here.

The molybdenum content of the basal lamb ration reported here (0.36 p.p.m.) seems quite large in comparison to the basal levels used in chick work (20 mcg. per kg.; Higgins *et al.*, 1956). Analysis of this ration prior to processing revealed that it was practically devoid of molybdenum (0.01 p.p.m.). The processing procedure (Ellis *et al.*, 1956) entailed considerable raking with a garden rake while the "cooked" ration dried on galvanized tin drying pans. This process evidently provided a greater portion of the basal ration's molybdenum content, and assumably, provided 0.35 p.p.m. to the supplemental molybdenum ration. No estimate could be made of the molybdenum and copper contamination obtained from the galvanized tin feeders.

On the basis of the well established relationships between molybdenum, copper and sulfate (Dick, 1957) it might be expected that the relative amounts of copper and sulfate would influence the effective level of molybdenum. The *in vitro* experiments (table 2) suggest a possible relationship of copper and molybdenum upon cellulose degradation.

Summary

Molybdenum and copper additions to rumen microorganism from alfalfa fed sheep promoted increased *in vitro* cellulose degradation to the same degree as alfalfa ash additions. Washed rumen organisms from alfalfa fed sheep contained about 1 p.p.m. molybdenum on a dry fat free basis. A semi-purified ration containing 0.36 p.p.m. molybdenum was formulated. A similar ration was supplemented with sodium molybdate to raise its molybdenum content to 2.36 p.p.m. In two feeding trials, lambs receiving the ration with added molybdenum made significantly faster gains than their controls. The addition of molybdenum significantly increased the digestibility of cellulose but did not significantly alter the digestibility of the other proximate ration constituents.

This work demonstrates a nutritional role for molybdenum in the growing lamb in that it is required for optimum cellulose digestion by the rumen flora. It further suggests that part of the beneficial effect of alfalfa ash for ruminants fed poor quality, high roughage rations resides in its molybdenum content.

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USE OF AN ESOPHAGEAL-FISTULA CANNULA FOR COLLECTING FORAGE SAMPLES BY GRAZING SHEEP

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MANY methods for collecting plant samples representative of forage material consumed by livestock have been devised. However most of these are subject to great personal error and can be considered at best only approximations.

Hand plucking plant material comparable to the forage eaten has been the most common method. This is believed satisfactory on stands of pure species but on complex mixtures it is totally inadequate (Cook *et al.*, 1951).

Harvesting protected and unprotected plots and using the differences as a measure of ingested material has been used widely on pasture mixtures (Hien *et al.*, 1937; Hodgson and Knott, 1942; Rhoad and Cook, 1937; and Richards and Hawk, 1945).

A similar method used by Cook *et al.* (1948) consisted of collecting specific plant units before grazing and again after grazing. The difference in weight and chemical composition between the two sets of samples served as a measure of ingested forage and nutrients.

It has often been stated that a representative sample could be obtained only by letting the animal forage it. This was recently accomplished in California by an esophageal fistula (Torell, 1954). This fistula in the esophagus was closed and opened by using stainless steel pins located on each side of the fistula.

Studies by Bath *et al.* (1956) showed that the chemical composition of the forage was changed very little by passing through the mouth and out an esophageal fistula into a collection bag. Therefore esophageal fistulated sheep can be used for collecting representative samples of ingested material in field digestion trials.

Methods and Results

In the present study esophageal-fistula cannulae were used and the forage samples were obtained by removing the screw cap on the outer end of the cannulae (figures 1 and 2).

A bag constructed of water-proof canvas was used to collect the forage sample as it was voided from the cannula. The bag was secured to the sheep with canvas straps fastened on the top of the neck (figure 3).

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Figure 1. Esophageal-fistual cannula installed with cap removed.

On October 25, 1956, four sheep were fitted with the type of fistula cannula shown in figure 3. Three of these sheep were used daily for eight months without incidence. One sheep out of the four lost its cannula because of necrosis two months after it was installed.

Two other types of cannulae were used on other sheep and were only moderately successful. One of these sheep died of pneumonia, two others lost their cannulae because of necrosis of esophageal tissue, and one broke the stem on the cannula when it caught in a woven wire fence.

The sheep equipped with esophageal-fistula cannulae were used to collect representative samples of ingested forage for chemical analyses. Other sheep were used in the experimental areas to collect liquid and solid excreta by means of collection bags (Cook *et al.*, 1951). The sheep with esophageal cannulae were allowed to run with the other sheep during the entire preliminary feeding period and digestion trial which required about two

Figure 2. Plastic esophageal-fistula cannula made of 1 inch lucite rigid tubing.

weeks. Trials were run continuously from October 1, 1956, until June 1, 1957.

The surgical procedure for the introduction of the plastic esophageal-fistula cannula is a delicate operation.

The animal is most conveniently anesthetized with a general anesthesia (pentobarbital sodium), then placed in right lateral recumbency and restrained by confinement of all limbs. The ventral cervical region is clipped free of wool and disinfected. A ventral skin incision approximately 6 inches in length is made in the middle one third of the cervical region. This incision is carefully extended through the subcutaneous fascia thence bluntly extended between the medial margin of the steron-cephalicus muscle and the lateral margin of the omohyoideus muscle. Care is taken to avoid the

Figure 3. Sheep grazing with collection bags attached to neck.

common carotid artery and vagosympathetic nerve trunk present at the right dorsal quadrant of the trachea.

The esophagus is isolated by blunt dissection and exposed to view. Identity of the esophagus may be enhanced by the passage of a male horse catheter via the nasal cavity. The esophagus is packed off from the surrounding tissue by sterile towels or sterile sponges. An incision approximately 3 inches in length is made longitudinally through the esophageal wall into the lumen.

The flanged end of the plastic-fistula cannula (figure 4) is inserted into the lumen of the esophagus. The anterior and posterior limits of the incision are closed by inverting the edges of the esophageal submucosa with 000 silk sutures. Next a purse-string suture is employed to hold the submucosa of the esophagus around the tubular portion of the fistula using a continuous pattern of No. 2 medium-chromic catgut, and the subcutaneous fascia is sutured using the same pattern and suture material. It is advisable to suture the subcutaneous fascia thus aiding in the support of the tubular portion of the fistula cannula. The skin incision is closed in a conventional manner. After-care consists of antibiotic and supportive therapy.

Before the installation of the cannula, all animals were adjusted to a pelleted feed. The pelleted material consisted of alfalfa and soybean oil meal. The pellets were one-fourth inch in diameter, commonly referred to as rabbit or poultry-sized pellets.

These pellets were fed two weeks prior to the operation and 1.5 weeks following the operation after which they were adjusted to alfalfa hay gradually for a few days. At the end of two weeks following the operation, the animals were used in the experimental trials to collect samples of forage.

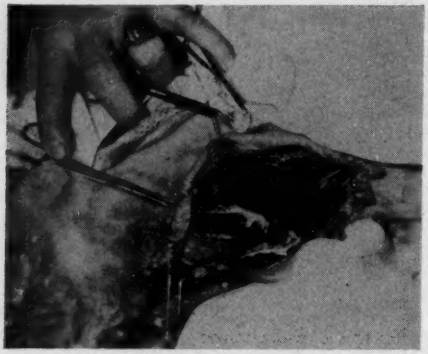

Figure 4. Installation of an esophageal-fistula cannula for collecting plant material.

Each evening while collecting forage samples, the sheep with fistula cannulae were placed in a pen. Early the following morning, they were equipped with collection bags around the neck and allowed to graze with the main herd. Under desert range conditions, it required about two to four hours for the grazing animals to fill the forage sample bag. On cultivated pastures it required considerably less time. The time required to obtain a sample depends on the individual sheep and the type and amount of forage present.

On desert ranges where the diet consisted of only a relatively few species, as much as 50 to 70% of the material collected from the cannulae could be identified taxonomically. Woody browse plants such as sagebrush (*Artemisia tridentata*) and shadscale (*Atriplex confertifolia*) could be almost completely identified, whereas grasses and herbaceous plants with woody bases were frequently masticated to the extent that they were too fine for identification.

During the winter grazing season, plant material in the sample could be distinguished frequently by its color. Black sage (*Artemisia nova*) had a dark green color and could be distinguished from winterfat (*Eurotia lanata*) which had a whitish-green color. Russian-thistle (*Salsola kali*)

had a black color and grasses in general had a light yellow color. Color differences were not so marked in the spring during the growing season.

The leaves of shadscale, winterfat, black sage, and fourwinged saltbush (*Atriplex canescens*) were quite easily identified in the samples. The leaves of grasses could be distinguished from those of shrubs, however, identification of individual grass species was difficult.

Seedheads were scarce on most species but those that were present in the sample could be readily identified.

On cultivated pastures, forbs could be distinguished from grasses. It was, however, difficult to distinguish one species of grass from another. This was likewise true for species of forbs but to a lesser extent.

Summary

A new technique for collecting herbage samples by means of sheep equipped with esophageal-fistula cannulae is described.

Several fistulated sheep were used to collect forage samples representative of ingested material. Other sheep were used in the experimental areas to collect liquid and solid excreta by means of collection bags. Thus by using the lignin-ratio procedure the digestibility of range forage can be determined.

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THE INFLUENCE OF ALFALFA HAY AND FRACTIONS OF ALFALFA HAY UPON THE DIGESTIBILITY OF OAT SILAGE [1, 2]

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THE increased use of poor quality roughages (corn cobs, corn stover, straw, and cottonseed hulls) in beef cattle rations has stimulated interest in supplements which will improve the nutritional status and performance of the animals receiving such low quality roughages.

Many experiments with beef cattle have shown that the addition of alfalfa meal will improve growth rate and reproductive efficiency. Beeson and Perry (1953) reported that the feeding of 0.5 lb. of dehydrated alfalfa meal per steer daily increased gain and feed efficiency above and beyond that which would be expected from the extra carotene, protein or energy furnished by this small amount of alfalfa meal. Bentley *et al.* (1952) reported that the average daily gains of steers receiving timothy hay, corn-and-cob meal, and soybean oil meal were significantly increased when the soybean oil meal was replaced by dehydrated alfalfa meal. Baker *et al.* (1947) improved performance of steers by adding alfalfa meal, at from 1 to 3 lb. daily, to a good quality ration. Because the protein content of the rations was equalized in these tests these workers suggested that the response was due to as yet unidentified factors not contained in their experimental rations.

In attempts to identify the factor(s) responsible for this observed stimulatory effect, several workers have shown that part or all of this stimulus is produced by the ash or mineral portion of the alfalfa meal; Klosterman *et al.* (1953), Burroughs *et al.* (1950), Chappel *et al.* (1955), Swift *et al.* (1951), and Tillman *et al.* (1954b). Conversely, other workers, Bentley and Moxon (1952) and Tillman *et al.* (1954a), have failed to obtain any response from adding alfalfa ash to poor quality rations.

The purpose of this study was to ascertain whether the addition of small amounts of chopped alfalfa to an oat silage ration would increase the digestibility of the silage and, if so, attempt to determine which of the major nutrient components in alfalfa are responsible for the increased digestibility.

[1] This paper is part of a thesis submitted by the senior author to the Graduate College, University of Illinois, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Science.

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Experimental Procedure

Animals Used. Six Hereford yearling steers, averaging 698 lb., were placed on a full feed of oat silage for a preliminary feeding period of 42 days. During that period they became accustomed to their individual feeding stalls and also established the level of oat silage that they would consume daily. Following the preliminary period the steers were assigned at random to the experimental rations shown in table 1. The design used was a 6 x 6 latin square in which each treatment preceded each other treatment only once.

TABLE 1. RATIONS

Treatment	Ration[a]	Pounds fed per steer daily
E	Oat silage	36.00
D	Chopped alfalfa hay	10.00
A	Oat silage	28.80
	Chopped alfalfa hay	2.00
B	Oat silage	28.80
	Cerelose	1.00
C	Oat silage	36.00
	Alfalfa ash	0.1436
F	Oat silage	28.80
	Soybean oil meal	0.76
	Cerelose	0.40

[a] Each steer was fed daily 0.22 lb. of a mixture of equal parts salt and dicalcium phosphate in each ration.

Experimental Methods. A preliminary feeding period of at least seven days was used prior to each collection period. This was followed by a 7-day collection period. During each preliminary feeding period the steers were fed twice daily in individual stalls. After they had consumed their feed, they were released into a sheltered paved area. Water and block salt were available at all times. All animals were on a constant feed intake prior to each collection period.

Feces were collected in metabolism stalls described by Horn *et al.* (1954). While in the stalls the steers were fed at 8:00 A.M. and 4:30 P.M. and watered once daily at approximately 10:00 A.M.

Throughout each collection period the fresh feces were removed from the collection boxes periodically throughout the day and placed in covered containers. Each morning the total 24-hour collection from each steer was weighed and thoroughly mixed. A 5% aliquot was then removed, placed in a plastic bag, and frozen. Following each 7-day collection period the seven aliquots from each steer were thawed, thoroughly mixed and sampled.

Following drying, the sample was ground and a proximate analysis conducted thereon according to A.O.A.C. Methods (1950).

Rations. The daily rations which were fed under each treatment are shown in table 1. The oat silage used in this study was cut while in the early dough stage (29.7% dry matter) and ensiled without a preservative. The oat silage contained 11.2% protein on a dry matter basis and was rated high in quality. The alfalfa was excellent quality first-cutting hay which contained 17.7% protein and was chopped into approximately 1-inch lengths prior to feeding. The ration shown under treatment E served as the basal ration for determining the oat silage digestibility when fed alone, while treatment D was used only as a means of establishing the digestibility of the chopped alfalfa hay which was fed. Using 36 lb. of oat silage as the basal full-fed ration, 2 lb. of chopped alfalfa hay dry matter were substituted for 2 lb. of oat silage dry matter. In the remaining three treatments the energy, protein, and ash equivalents of 2 lb. of alfalfa hay were fed with the oat silage. The 1 lb. of cerelose fed in treatment B replaced the 1 lb. of estimated TDN which would have been derived from 2 lb. of alfalfa hay. In treatment C, alfalfa ash equivalent of 2 lb. of alfalfa hay was fed. For treatment F, 0.76 lb. of soybean oil meal was used to replace the protein equivalent of the alfalfa hay. Enough cerelose was also added to balance the energy equivalent of 2 lb. of alfalfa hay.

A representative daily feed sample of each ration was taken three days prior to and during each collection period. The resulting composited sample was dried, ground, and later subjected to a proximate analysis according to A.O.A.C. Methods (1950).

Digestion coefficients were calculated on all rations and subjected to an analysis of variance as outlined by Snedecor (1946). In calculating the apparent digestibility of the rations the digestibility of the cerelose was assumed to be 100%. Since no soybean oil meal was fed alone its digestion coefficients were taken from the compilation of Schneider for cattle (1947). The coefficients used were 88, 90, 57, 38 and 91% for organic matter, crude protein, crude fiber, ether extract, and NFE respectively.

Results and Discussion

The means and standard deviations of the digestion coefficient for organic matter, crude protein, crude fiber, ether extract and NFE are shown in table 2. These coefficients are expressed as an average of the 6 steers which were subjected to each treatment. When the individual coefficients were subjected to statistical analysis the only significant differences resulting were the higher digestibilities of the organic matter and crude protein and the lower digestibility of the ether extract in the chopped alfalfa hay control ration. These differences were significant at the 1% level. Inasmuch as this ration (treatment D) was used solely as a positive

control for the indirect determination of the oat silage digestibility in the oat silage-alfalfa hay ration (treatment A), its significantly higher digestibility has little relevance to this study.

Since neither alfalfa hay nor any of its component parts enhanced the digestibility of the oat silage, the ration consisting of oat silage and the simple mineral mixture fed apparently contained sufficient balance and quantity of the nutrients studied to promote optimum microbial activity in the paunch. On the basis of these results as well as those reported in other experiments by Staheli (1956), it appears that a ration of good

TABLE 2. APPARENT DIGESTION COEFFICIENTS, AVERAGE OF SIX STEERS PER TREATMENT

Treatment	Organic matter	Crude protein	Crude fiber	Ether extract	NFE
	%	%	%	%	%
E Oat silage	60.70 ±2.51	61.98 ±1.14	58.90 ±1.14	69.59 ±3.03	59.00 ±1.85
D Chopped alfalfa hay	68.24** ±2.30	77.99** ±2.25	58.62 ±2.97	43.28** ±5.55	72.36** ±2.01
A Oat silage+chopped alfalfa hay	58.38 ±2.60	58.73 ±1.93	55.88 ±3.08	68.99 ±3.87	56.50 ±3.04
B Oat silage+cerelose	60.15 ±2.88	57.86 ±2.20	59.68 ±2.83	72.08 ±3.49	58.33 ±3.75
C Oat silage+alfalfa ash	61.99 ±2.08	61.66 ±2.16	61.31 ±2.34	72.65 ±3.70	58.58 ±2.42
F Oat silage+soybean oil meal and cerelose	59.87 ±1.23	59.03 ±1.67	59.87 ±1.17	69.46 ±3.23	57.88 ±1.43

** Significantly different from all other treatments ($P<.01$).

quality oat silage and a calcium-phosphorus mineral mixture needs only additional energy and in some cases additional protein to render it a complete and satisfactory ration for beef cattle.

Summary

A series of balance studies were conducted to determine the influence of alfalfa hay and fractions of alfalfa hay upon the digestibility of oat silage. Six Hereford yearling steers were used in a 6 x 6 latin square design with each steer receiving each of the six treatments. Apparent digestion coefficients were determined for the organic matter, protein, crude fiber, ether extract and N.F.E. of the oat silage.

The results showed that the addition of 2 lb. of alfalfa hay or its equivalent in energy, protein, or ash to a ration consisting of oat silage and a calcium-phosphorus mineral mixture did not increase the digestibility of the oat silage.

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THE EFFECT OF STAGE OF MATURITY AND LIGNIFICATION ON THE DIGESTION OF CELLULOSE IN FORAGE PLANTS BY RUMEN MICROORGANISMS *IN VITRO* [1, 2]

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THE stage of maturity of forage plants at the time of harvest has a marked influence on their feeding value and digestibility for beef cattle (Moxon *et al.*, 1951) and dairy cattle (Trimberger *et al.*, 1955). The conventional proximate analysis procedure is inadequate for measuring the changes which take place in maturing forage plants and which affect the digestibility of the nutrients in these feeds. This weakness in the proximate analysis results is emphasized by the fact that the apparent digestibility of the nitrogen-free extract fraction in roughages may be lower than the apparent digestibility of the crude fiber fractions (Moxon *et al.*, 1951). A considerable portion of the lignin in roughages is accounted for in the nitrogen-free extract as determined by the proximate analysis procedure (Moxon and Bentley, 1953).

Since lignin is not digested by ruminants (Crampton and Maynard, 1938; Gray, 1947) and the lignin content of forage plants increases with maturity, changes in this constituent have been considered as a partial explanation for poor feeding value of mature forages. Among the results supporting such a conclusion are these: (1) Prefeeding chemical treatment of roughage designed primarily to remove the lignin has been shown to increase the digestibility of roughages by ruminants, as demonstrated by Lehmann, 1891; Honcamp, 1919; Beckman, 1923, and many other workers. (2) Microscopic examination of cellulose fibers in the process of being digested, suggested to Baker and Harriss (1947) that lignification is a physical barrier to cellulose digestion by cellulolytic rumen micro-organisms. Thus it would appear that lignin might influence digestibility by acting as an "encrusting substance" and thus preventing the digestive enzymes of the rumen micro-organisms and of the intestinal juices from attacking the plant nutrients.

The development of the "artifical rumen" or *in vitro* technique for the study of rumen fermentation (Burroughs *et al.*, 1950, and Bentley *et al.*,

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[3] This work was taken in part from a dissertation presented by L. D. Kamstra to the Graduate School, The Ohio State University, in partial fulfillment of the Ph.D. degree in 1955. Present address is Animal Husbandry Department, South Dakota State College, Brookings, South Dakota.

1954) seemed to offer a method of studying the relationship of lignin as an "encrusting substance" to the digestibility of forage samples, and, in fact, a method for estimating cellulose digestibility of forages. The digestion of three different forages harvested at three different stages of maturity and of cellulose and holocellulose prepared from the same forage samples has been determined in the artificial rumen. The results of these studies are reported herein.

Experimental

The comparative digestibilities *in vitro* of cellulose as it occurs naturally in various plant materials and of cellulose isolated from these same plant materials was determined by an adaptation of the rumen fermentation technique, as described by Bentley *et al.* (1954). A cellulose-containing fraction (holocellulose) was prepared from several of the same plant materials and digestibility of its cellulose compared with the cellulose samples by the *in vitro* fermentation technique.

A mature Angus steer, fitted with a permanent rumen fistula and maintained on good quality alfalfa hay, served as the source of the rumen micro-organisms for all of the *in vitro* fermentations. Rumen juice was strained through four layers of cheesecloth, passed through an electrically driven Sharples supercentrifuge and the sediment collected on a celluloid liner fitted inside the centrifuge cylinder. The sediment from one liter of rumen juice was resuspended with the aid of a loose-fitting Potter-Elvehjem homogenizer in 250 ml. of buffer, pH 7.0. The buffer contained 1.059 gm. Na_2HPO_4, 0.436 gm. KH_2PO_4 and 100 mg. cysteine hydrochloride per liter. Each fermentation flask was inoculated with 10 ml. of the micro-organism suspension.

The pH of the flask contents was adjusted to 6.9 at the beginning of the experiment by the addition of 2M Na_2CO_3. This process was repeated at two to four hour intervals during the first 12 to 16 hours of the fermentation period and again after 24 hours to adjust and maintain the pH of the fermentation mixture between 6.7 and 6.9.

The basal medium was similar to that described by Bentley *et al.* (1954), with the addition of 0.02 mg. of biotin, 0.05 mg. para-aminobenzoic acid and 10 mg. valeric acid per 100 ml. of medium.

Cellulose was determined by the gravimetric method of Crampton and Maynard (1938). Cellulose was isolated from the various forage samples by the same procedure except that the final product was collected on a 200 mesh silk bolting cloth instead of in a Gooch crucible. Holocellulose, which contains both cellulose and hemicellulose but has a low lignin content, was prepared from the forage samples by the method of Whistler *et al.* (1948). The lignin content of the plant materials was determined by the method described by Patton (1943).

Each forage variety was harvested in 1955 at three different stages of maturity. First, second and third stages of orchard grass represented leafy or shooting stage, pre-bloom stage, and late bloom stage, respectively. First, second and third stages of alfalfa represented leafy stage, early bloom stage and late bloom stage, respectively. First, second and third stages of timothy represented leafy or shooting stage, early bloom stage and late bloom stage, respectively. The samples were cut by hand and separated from other plants. They were dried soon after cutting in a forced-draft oven at 65° C. and ground to 40 mesh with a Wiley mill. In 1956, alfalfa samples were harvested at stages quite similar to those described for the 1955 samples and in addition, one sample was obtained at an earlier stage, leafy, 6 to 8 inches tall.

TABLE 1. DIGESTION OF CELLULOSE IN FORAGES AS RELATED TO THE AMOUNT OF CELLULOSE IN EACH FERMENTATION FLASK AND STAGE OF MATURITY OF THE FORAGE

Grams cellulose added per flask[a]	Cellulose digested in 30 hours			
	First stage alfalfa		Third stage alfalfa	
	gm.	%	gm.	%
.10	.08	80.0	.04	40.0
.30	.26	86.7	.14	46.7
.50	.42	84.0	.25	50.0
.80	.67	83.7	.40	50.0

[a] Sufficient plant material added to each flask to supply the amount of cellulose indicated.

Results

In a series of preliminary experiments it was observed that the amount of cellulose in the flasks affected the amount of cellulose digested (table 1). This is an important consideration since it is necessary to establish that the amount of cellulose digested by the microflora in the fermentation flasks is dependent on the amount of available cellulose in the flask. Furthermore, the preliminary results in table 1 show that maturity of the forage affects the *in vitro* digestion of the cellulose in the plant material. The percentage digestion and the amount of the alfalfa cellulose digested in 30 hours are given for two alfalfa samples collected at early and late stages of growth. With the first stage alfalfa, an average of 83.6% of the cellulose was digested, whereas only 46.7% of the cellulose in the third stage alfalfa was digested. These differences in the amount of cellulose digested in the samples representing forages harvested at different stages of maturity as well as the obseration that cellulose digestibility *in vitro* seemed to reflect the amount of available cellulose, suggested that the

in vitro technique might be a useful tool for estimating the digestibility of cellulose in forages.

Digestion of Whole Plant Cellulose and Isolated Cellulose. A more extensive study was then undertaken and the *in vitro* digestion results for cellulose in whole plant material and cellulose isolated from the same plant materials and the cellulose, holocellulose and lignin content of the whole plant material are given in table 2. It is readily apparent from these results that the stage of maturity of the plant at harvest had a marked effect on the *in vitro* digestibility of the cellulose in orchard grass,

TABLE 2. CELLULOSE, HOLOCELLULOSE AND LIGNIN CONTENT AND *IN VITRO* DIGESTION OF CELLULOSE IN THE PLANT MATERIAL AND CELLULOSE ISOLATED FROM THE PLANT MATERIAL

Plant material	Harvest date (1955)	Composition			*In vitro* digestibility [a]		
		Cellulose	Holocellulose	Lignin	Cellulose (Whole plant)	Cellulose isolated	Holocellulose
		%	%	%	%	%	%
First stage orchard grass	April 21	32.8	66.2	8.58	77.6	96.0	90.1
Second stage orchard grass	May 11	37.2	71.0	9.96	74.4	....	87.2
Third stage orchard grass	June 6	34.9	71.2	14.39	31.9	86.4	73.6
First stage alfalfa	April 21	28.2	53.5	7.85	84.1	82.2	81.4
Second stage alfalfa	May 24	32.4	65.2	11.30	60.6	....	67.1
Third stage alfalfa	June 20	31.4	68.3	12.9	38.4	60.1	65.7
First stage timothy	April 21	33.9	62.3	9.65	77.5	86.0	83.1
Second stage timothy	May 24	37.0	65.8	11.76	66.9	....	78.8
Third stage timothy	June 20	36.2	72.3	13.58	48.0	73.3	67.8
Alfalfa leaves, second stage	May 11	17.8	53.7	5.7	67.8	83.4	79.6
Alfalfa stems, second stage	May 11	41.5	72.9	18.5	44.4	80.2	71.5
Corn cobs		42.1	81.9	14.34	19.9	86.0	70.1
Solka-Floc		92.0	....	.....	60.9	62.0	....
Oat hulls		33.7	....	17.7	Less than 10	86.2	....
Wheat bran		....	....	7.26	Less than 10	74.0	....

[a] Thirty-hour fermentations and 0.3 gm. of cellulose in whole plant or isolated cellulose and 0.2 gm. of cellulose in holocellulose per flask were used. Each value for digestibility represents an average of three separate experiments.

alfalfa and timothy. However, when cellulose isolated from these same plant materials was used as the cellulose substrate in the fermentation flasks, the digestibility was found to be improved over the corresponding sample of whole plant material.

Isolated cellulose, in general, showed less variation in digestibility, *i.e.*, as affected by stage of maturity and different plant sources than did the cellulose in whole plant materials. The isolated cellulose was also much more readily digested than the cellulose in the whole plant material. No appreciable digestion of the cellulose in oat hulls or wheat bran occurred during a 30-hour fermentation, but, as with the forage samples, cellulose isolated from the hulls or the bran was readily utilized by the microorganisms. These results (table 2) would suggest that "encrusting substances" could be involved since the isolated cellulose would be free of lignin or other "encrusting substances" indigenous to the whole plant

materials. The results with oat hulls and bran also indicate the possibility that the distribution of the lignin in the plant may be important. The lignin content of wheat bran is lower than that of any of the hay samples yet the cellulose in bran is not as digestible as in the mature hay samples of much higher lignin content.

Lignin *per se* does not appear to influence the activity of rumen organisms *in vitro*. Lignin was isolated from a sample of full-bloom alfalfa hay by drying the residue from the procedure for lignin determination. Some of this dried lignin preparation was added back to the fermentation flasks containing cellulose and had no effect upon the amount of cellulose digested. It would appear that this experiment lends more support to the theory

TABLE 3. THE CRUDE FIBER, CELLULOSE, LIGNIN AND PROTEIN CONTENT OF THE 1956 ALFALFA SAMPLES USED AND THE *IN VITRO* DIGESTION OF CELLULOSE IN THE SAMPLES

	Harvest date (1956)	Crude fiber, A.O.A.C.	Cellulose	Lignin	Protein	Cellulose digestion[a]
		%	%	%	%	%
Leafy alfalfa, 6 to 8 inches tall	May 8	12.06	10.97	4.86	28.55	71.1±4.5 (5)[b]
First stage alfalfa	May 18	20.36	18.86	8.20	22.89	58.7±7.7 (8)
Second stage alfalfa	June 23	32.38	28.55	10.75	16.68	52.0±8.6 (8)
Third stage alfalfa	July 17	33.94	29.44	12.03	14.18	47.3±9.3 (8)

[a] Each fermentation flask contained 0.379 to 0.414 gm. of cellulose per 100 ml. of fermentation medium supplied by varying amounts of whole plant material.

[b] Average cellulose digestion with average error of the mean based on 30-hour fermentation results. Number in parenthesis indicates number of values used in compiling average.

that the lignin inhibits cellulose digestion by an encrusting action based upon the deposition of lignin around the cellulose during the growth of the plant.

In 1956, alfalfa samples were again harvested at growth stages comparable to those described for the 1955 samples. The lignin, cellulose and crude fiber contents and the results of *in vitro* digestion experiments are given in table 3. A comparison of the cellulose and lignin contents of the 1955 alfalfa samples to those found for the same constituents in the 1956 samples reveals that the samples were quite different. The 1955 samples contained more cellulose and lignin. The composition of the plants may have been influenced by the season as the 1956 season was later, cooler and wetter than in 1955.

The *in vitro* results show that lowered cellulose digestion was obtained with the more mature samples, although the spread between the early versus the late stage was less marked for the 1956 alfalfa samples than in 1955 (table 2 vs. table 3).

Since one important purpose of this paper is to consider fundamental problems affecting the validity of the use of *in vitro* rumen fermentation in the determination of forage cellulose digestibility, it is of interest to

consider the rate of digestion during the fermentation. In figure 1, the digestion results are given for samples taken from the fermentation flasks at various time intervals during the fermentation. The most rapid cellulose digestion occurred between the 7th and 12th hours of the fermentation; however, by the 24th hour the disappearance of cellulose for each of the forages began to slow down so that between the 24th and 30th little

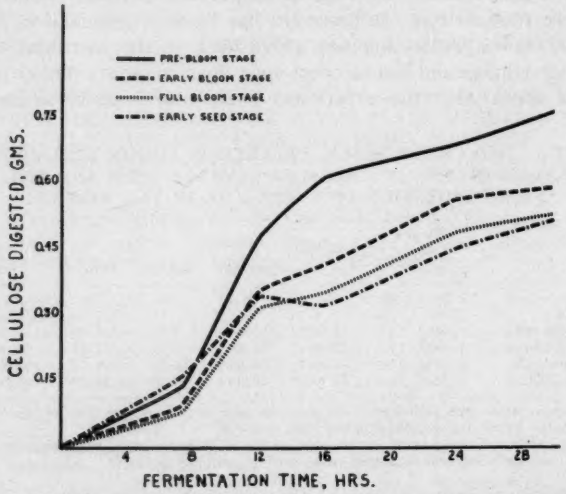

Figure 1. The digestion of alfalfa cellulose (whole plant) *in vitro* as influenced by fermentation time. The results reported here as gram cellulose digested were based on analyses of aliquots removed from the fermentation flasks as 8, 12, 16, 24 and 30 hours after the start of the experiment. An excess of cellulose, 1.14 to 1.24 gm., from the various plant materials was present in each flask.

cellulose was digested. This suggests that by the 24th hour the digestion of available cellulose had nearly reached completion, as lengthening the fermentation time did not alter the amount of cellulose digested in a given forage sample appreciably. The importance of these results is that an "end point" for the fermentation can be established and that the digestibility of cellulose as determined under standardized conditions, should be a measure of cellulose availability.

Discussion

The objective of this paper has been to present experimental results which suggest that the extent of digestion of cellulose in an *in vitro* rumen fermentation appears to be related to the availability of the cellulose to

the organisms attacking it. For such an approach to be valid, it must be demonstrated that adding increasing increments of cellulose or whole plant material (substrate) to the fermentation flasks results in proportionately more cellulose being digested. From the results of table 1 it appears that this relationship exists. To test the hypothesis, a number of common forage plants were harvested at widely different stages of maturity during two different years and the digestibility of the cellulose contained therein determined. Cellulose availability (digestion), as measured *in vitro*, varied indirectly with maturity of the plant at harvest, the most mature being the least digestible. Cellulose isolated from the various forage samples was more digestible than the cellulose as it existed in the plant. This observation, coupled with the increased lignin content of the more mature plant materials, suggested that the amount and/or distribution of lignin contained in the plant was responsible for part of the decrease in cellulose availability. These observations would conform to those reported on the effect of maturity of forages on their feeding value and digestibility *in vivo* (Norman, 1953; Crampton, 1939; Patton, 1943; Dyer *et al.*, 1954; Phillips *et al.*, 1942; Ely *et al.*, 1953; Trimberger *et al.*, 1955) and the effect of lignin on the digestion of fibrous plant material (cellulose) *in vitro* (Baker and Harriss, 1947; Howie and Baker, 1952).

No attempt is made to give the results on estimates of *in vitro* cellulose digestion values in this paper but rather to report the results of experiments pointing to the feasibility of using the artifical rumen technique for this purpose. Further work with samples of forages on which digestibility has been determined by conventional methods is needed in an effort to establish a correlation between the *in vitro* and the *in vivo* values. Kamstra (1955) in a preliminary experiment, found that in a 24 hour *in vitro* digestion period an orchard grass-alfalfa mixture, a pelleted feed mixture, and a corn and cob meal-poor hay mixture showed cellulose digestions of 60.33, 48.00 and 31.66%, respectively. These same feeds showed *in vivo* digestion coefficients with cattle of 68.17, 46.10 and 41.61, respectively. The concept of establishing the relationship between the amount of available substrate and microbial activity and growth has been reported by Hershberger (1955; 1955a) for protein synthesis from nonprotein nitrogen present in the medium. Pigden and Bell (1955) demonstrated that TDN values obtained after a forty-eight hour fermentation (*in vitro*) using eleven forages as substrates, compared favorably with values obtained *in vivo* with sheep.

In the comparison of digestibility of cellulose, as present in the whole plant versus the cellulose isolated from the same plant, it is shown that the cellulose fraction itself differs in digestibility from one stage of plant growth to another and from one species to another. Many workers postulate reasons for differences in cellulose digestion; Karrer and Schubert

(1924), working with snail secretions, showed that the amount of cellulose breakdown was inversely proportional to the degree of crystallinity of the cellulose fraction. Perlin *et al.* (1947) and Norkrans (1950) using cellulytic micro-organisms confirmed Karrer's observation and further showed that by changing the crystalline cellulose to amorphous cellulose they increased the rate of microbiological decomposition of the cellulose. Ranby and Immergot (1955) have demonstrated that physical constants are not identical for cellulose from different sources.

The data do suggest a closer relationship between the digestibility of isolated cellulose samples than exists between the cellulose in samples of the whole plant materials, but do not necessarily indicate a general relationship of all celluloses regardless of source or stage of maturity. The higher and more uniform digestibility of isolated cellulose could be related to removal of lignin more than other cell-wall constituents since the hemicellulose in the holocellulose fraction did not inhibit cellulose digestion from the fraction. The holocellulose fraction was digested in the same order of magnitude as isolated cellulose.

It appears that lignin is one of the main single components of the plant which tends to decrease cellulose digestion by micro-organisms. Baker and Harriss (1947) and Howie and Baker (1952) demonstrated the manner of attack by microflora upon cellulose. They made photomicrographs showing bacterial attack at points of fracture or breaks in the fiber. Thus, it is not unreasonable to suggest that lignin, because of its close association with cellulose, could substantially inhibit microbial attack on cellulose particles. It must not be assumed, however, that lignin is the only factor in decreasing cellulose digestion from the whole plant since it was observed that the most lignified plant did not always have the lowest cellulose digestibility. Within any one plant species studied, however, the most lignified plant stage did show the lowest digestibility.

Summary

The *in vitro* digestibilties of cellulose within various whole plant materials and cellulose-containing fractions (isolated cellulose and holocellulose) were determined by an *in vitro* technique. Plant materials at different stages of maturity were investigated: first, second and third stages of orchard grass, alfalfa and timothy. Lignin content was correlated with the digestion of cellulose within the whole plant materials.

The results of the investigation showed that separating cellulose from lignin greatly improved its digestibility *in vitro*. However, cellulose in holocellulose, which also contains hemicellulose, was similar in digestibility to isolated cellulose. This would indicate that lignin but not hemicellulose has an inhibitory effect upon cellulose digestion.

Maturity of plant material has an effect upon cellulose digestion—the young plant cellulose being the more digestible. The effect of maturity

diminished greatly when the cellulose was isolated from the plant, either as holocellulose or as cellulose. A negative relationship existed between lignin content and the *in vitro* cellulose digestion in the whole plant materials.

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THE RESPONSE OF RUMEN MICROORGANISMS TO PASTURE GRASSES AND PRICKLY PEAR CACTUS FOLLOWING FOLIAR APPLICATION OF UREA [1]

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THE nutritional quality of forage grasses depends primarily on the degree of maturity attained by the grass at the time of grazing or harvest. As maturity progresses, feeding quality is lowered. This has been demonstrated by feeding and digestibility experiments, and supported by analyses (Morrison, 1948).

The objective of these tests was to determine whether foliar application of urea to typical pasture grasses would improve their nutritional quality. An artificial rumen technique was used for these evaluations in view of data (Kamstra *et al.*, 1955; Pigden and Bell, 1955) demonstrating the applicability of *in vitro* techniques in assessing roughage quality. An *in vivo* rumen technique was also used in these tests to supplement the values obtained *in vitro*, as well as to provide protein digestibility data on the test forages.

Experimental

In Vitro Rumen Technique. Fermentations, *in vitro*, with mixed cultures of rumen microorganisms were carried out in Goetz (oil centrifuge) tubes of 100 ml. capacity. All samples of forage were run in triplicate. Each tube was charged with 1.0 gm. of dry roughage and 0.3 gm. cane molasses ash, which served as a source of trace minerals. A nutrient salt solution (Bentley, 1952) was also added, which contributed the following to each tube: mono-sodium phosphate, 72.3 mg.; disodium phosphate, 60.6 gm.; sodium bicarbonate, 102.3 mg.; potassium chloride, 14.5 mg.; sodium chloride, 14.5 mg.; magnesium carbonate, 1.7 mg.; sodium sulfate, 8.3 mg.; calcium chloride, 1.7 mg.; and ferric chloride, 2.45 mg. The calcium chloride and ferric chloride were kept in a stock solution separate from the other salts to avoid salt precipitation. Each tube was then inoculated with 25 ml. of strained and centrifuged steer rumen contents, collected before the morning feeding. The rumen ingesta, strained through a coarse muslin to remove the coarse feed particles, was centrifuged at 1000 rpm. for five minutes in 200 ml. centrifuge tubes to remove the finer feed particles, which would affect the results of a 24-hour fermentation.

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[2] The authors gratefully acknowledge the cooperation of the King Ranch, Inc., Kingsville, Texas, in these studies.

The cellulose content of the inoculum after centrifugation ranged between 0.004 and 0.008% in the various experiments. The volume of the mixture in the oil tubes was then adjusted to 50 ml. with distilled water and a drop of mineral oil was added to prevent frothing losses. The tubes were placed in a 39° C. constant temperature bath and carbon dioxide bubbled through the system via a short piece of tapered glass tubing extending into the stem portion of the oil tube, almost to the tip. This assured the necessary anaerobic conditions and agitation within the system. This tapered bubbling tube was held in place by a snug-fitting piece of stiff polyethylene (3 cm. diameter x 1 mm. thick). Polyethylene bottle cap liners can be used effectively for this purpose. The stream of carbon dioxide from the tip of the bubbling tube up the narrow stem of the Goetz tube plus the displacement of the bubbling tube itself prevents settling out of any of the insoluble ingredients during the course of the fermentation.

After 24 hours of fermentation, the tubes were centrifuged at 2000 rpm. for 15 minutes. The supernatant liquid was then decanted for analysis of residual ammonia by the A.O.A.C. method. Residual or undigested cellulose was determined by the method of Crampton and Maynard (1938) on the solid material left in the tubes.

In Vivo Rumen Technique. The principle of this technique is to expose the dry forage to the digestive action of the microbial population in the rumen of a rumen-fistulated steer for a 24-hour period and then determine the extent of the digestion which has occurred. To accomplish this, 20 to 30 gm. samples of chopped dry grass or diced dry cactus contained in rectangular "Dacron" cloth pouches, measuring 5 x 7 in. were placed in the rumen of a steer, fasted 18 hours. The "Dacron" cloth used for the pouches was approximately 100 mesh to minimize the exit of undigested forage particles from within the pouch and also the entrance of suspended rumen matter into the pouch without interfering with the free flow of rumen liquid. A rectangular frame made from 10 gauge stainless steel wire was inserted into each test pouch to prevent passage of the pouch to lower portions of the digestive tract. All samples of forage were evaluated in triplicate. No feed was given to the animal while the pouches remained in the rumen, thus minimizing the variables affecting the digestion of the forage under test. The pouches with the residual forage, upon removal from the rumen were rinsed in two changes of distilled water to remove the rumen liquid and adhering particulate matter. The pouches were then dried and weighed to obtain dry matter digestion values. The contents of each triplicate set of pouches were pooled because of the limited amount of residual forage for protein and cellulose analysis.

The ration fed to the steers used in these studies consisted of a mixture of clover and orchard grass hay along with a 13% protein supplement containing barley, soybean oil meal, oats, alfalfa meal, corn and cob meal and a mineral mixture.

Foliar Treatment of Prickly Pear Cactus. In the laboratory experiments, uniform cactus pads were dipped in urea solutions containing 50 and 200 lb. of urea plus a combination wetting and adhesive agent[3] per 100 gal. for 3 to 5 seconds. The control cactus pads were dipped into distilled water for the same period of time. All pads were suspended in air to permit their surfaces to dry. Some were washed with distilled water after eight hours and others were washed after 24 hours. The cactus pads were diced and dried in an air oven at 150° F. for 24 hours, prior to analysis and evaluation.

In a field test, urea was sprayed on a "white" variety of cactus at the rate of 160 lb. per acre contained in 40 gal. of water with "spreader-sticker". The control area received in equivalent amount of water and "spreader-sticker" as a spray. Samples were harvested seven days after spray treatment, diced and dried in preparation for analysis and evaluation.

Foliar Treatment of Pasture Grass

Pangola Grass. Urea was applied at rates of 10, 20, and 40 lb. per 100 gal. of solution with "spreader-sticker" per acre to a pangola grass pasture in Florida during December. Seventy-two hours after foliar application, grass samples were harvested from each plot and air dried.

Orchard-Fescue Grass. Urea was applied at rates of 40 and 80 lb. per 100 gal. of solution with "spreader-sticker" per acre to a grass pasture, predominantly orchard and fescue, in Pennsylvania during August. Samples were harvested from the test plots, as well as from the control plots 5 and 10 days after foliar application of urea. These samples were dried at 150° F. for 24 hours in preparation for analysis and evaluation.

Results and Discussion

The tabular data on the *in vivo* and *in vitro* evaluations of forages are the findings of individual experiments, all of which had been replicated two or three times for confirmation. The data of all experiments in each series were not pooled because of the observed variations in the plane of rumen activity from day to day, as well as from animal to animal.

The initial experiments were carried out with prickly pear cactus, a forage for cattle in the arid southwest regions of this country. As a forage, the prickly pear cactus supplies the animal with valuable carbohydrates and water, but is deficient in nitrogen when used as the sole feed. It was believed that a foliar application of an aqueous solution of urea would correct this nitrogen deficiency and thus bring the nutrients in better balance for improved animal performance.

The analytical data (table 1) clearly show that the nitrogen content of the cactus pads was increased by immersion into urea solutions. It was

[3] Du Pont "Spreader Sticker".

TABLE 1. NITROGEN CONTENT OF PRICKLY PEAR CACTUS FOLLOWING FOLIAR UREA APPLICATION

Cactus sample no.	Urea solution	Water wash	Nitrogen content[a]
	lb./100 gal.		%
1	0	None	1.3
2	0	None	1.0
3	0	None	1.2
4	50	None	1.4
5	50	None	1.7
6	200	None	2.1
7	200	None	1.3
8	200	After 8	2.2
9	200	hours	1.6
10	200	After 24	1.6
11	200	hours	1.6

[a] Dry matter basis.

also evident that most of the urea nitrogen was absorbed within eight hours, since washing did not effect the increased nitrogen content. All the urea-treated samples except one had a greater nitrogen content than any of the controls. The average nitrogen content of the treated pads was 1.8% or 50% greater than that of the controls, while the highest of the treated samples had a nitrogen content 83% greater than the controls. Evaluation *in vitro* of representative samples as the sole nitrogen and energy sources revealed that the urea-treated cactus samples showed significantly greater cellulose digestion rates than did the corresponding controls (table 2). In

TABLE 2. *IN VITRO* RUMEN RESPONSE TO PRICKLY PEAR CACTUS FOLLOWING FOLIAR UREA APPLICATION

Cactus sample no.	Urea in dip solution	Nitrogen[a] content	Cellulose[c] digestion
	lb./100 gal.	%	%
2	0	1.0	37.5
2+12 mg. urea[b]	0	1.0	38.4
3	0	1.2	39.7
5	50	1.7	58.9**
6	200	2.1	46.0*
8	200	2.2	61.9**
9	200	1.6	59.3**
10	200	1.6	54.3**

[a] Dry matter basis.
[b] Urea added (12 mg.) to fermentation system to equalize the total nitrogen content with that containing sample No. 8.
[c] Average standard deviation ± 1.9.
* Significantly different from untreated cactus $P<.05$.
** Significantly different from untreated cactus $P<.01$.

contrast to cellulolytic values of 37.5 and 39.7% for the controls, values for the cacti dipped in urea solutions ranged from 46.0 to 61.9%, with an average of 56.1%. Furthemore, supplementation of control samples with urea, to equalize the nitrogen in the test system with that containing the treated sample (No. 8) with the highest nitrogen content, failed to increase its rate of cellulose digestion. This would indicate that the metabolic effects of urea application plus the resulting nitrogen increases, rather than increased nitrogen alone, were responsible for the improved cellulolytic response.

Subsequent field tests demonstrated (table 3) that foliar urea applica-

TABLE 3. ANALYSIS AND *IN VITRO* AND *IN VIVO* RUMEN PERFORMANCE OF PRICKLY PEAR CACTUS SEVEN DAYS FOLLOWING FOLIAR UREA APPLICATION

Item	Cactus plot 1	Cactus plot 2
Urea applied, lb./acre	0	160
Nitrogen content, %[a]	1.44	1.73
Ammoniacal nitrogen content, %[a]	0.08	0.09
Urea content, %[a]	0.0	0.0
Cellulose content, %[a]	16.9	17.0
Cellulose digested, *in vitro*, %[b]	42.5	47.5[c]
In vivo:		
Cellulose digested, %	33.0	39.5
Dry matter digested, %[b]	48.9	57.4[c]
Digestible protein/100 lb. of dry cactus, lb.	0.27	4.14

[a] Dry matter basis.
[b] Average standard deviation ±1.4.
[c] Significantly different from cactus from the control plot $P<.05$.

tion of 160 lb. per acre was effective in increasing the nitrogen content of "white" prickly pear cactus from 1.44 to 1.73%. The minimal ammonia values and the absence of residual urea was indicative of complete conversion of absorbed urea.

In the laboratory rumen, the cellulose digestion rate of the urea-treated cactus was 12% greater than that of the control sample (table 3). Subsequent *in vivo* tests on the same samples confirmed these *in vitro* findings. Dry matter and cellulose digestion rates of the urea-sprayed cactus were approximately 20% greater than the untreated control. Digestible protein was increased from 0.27 to 4.15 lb. per 100 lb. of dry cactus.

On the basis of these findings, experimental plots of pangola grass in Florida were given a foliar application of urea at rates of 10, 20, and 40 lb. per acre. Three days later, the grass samples from each plot were harvested and air dried. Pangola grass treated with urea showed small increases in total nitrogen, but no residual urea, indicating complete con-

version of absorbed urea nitrogen (table 4). The cellulose content of all samples ranged from 34.7 to 36.0%.

Similarly, the urea-treated pangola grass showed improved performance in the laboratory rumen over the grass from the control plot (table 4). As little as 10 lb. of urea per acre resulted in grass which showed 37.1% cellulose digestion in comparison to 27.7% cellulose digestion for the control sample, or a 34% increase. Samples from plots which received 20 and 40 lb. of urea per acre gave cellulose digestion values of 40.6 and 42.0%, respectively. Where excess urea was added, the rate of cellulose digestion for both the control and the urea-treated samples was further

TABLE 4. ANALYSIS AND *IN VITRO* PERFORMANCE OF PANGOLA GRASS FOLLOWING FOLIAR APPLICATION OF UREA

Item	Grass plot			
	1	2	3	4
Urea applied, lb./acre	0	10	20	40
Nitrogen content, %[a]	1.25	1.50	1.50	1.50
Urea content, %[a]	0.0	0.0	0.0	0.0
Cellulose content, %[a]	34.7	35.4	36.0	35.0
Cellulose digested, %[b]	27.7	37.1**	40.6**	42.0**
Cellulose digested in the presence of excess urea (40 mg.), %[b]	44.9	54.0**	54.3**	55.7**

[a] Dry matter basis.
[b] Average standard deviation ±0.6.
** Significantly different from grass from the control plot $P<.01$.

increased (table 4). However, even under these conditions, the cellulolytic rate of the urea-treated grass samples ranged from 54.0 to 55.7% in contrast to 44.9% for the control. This is believed further proof of the quality difference between control and the urea-treated grass.

It is clear that the small increase in total nitrogen resulting from foliar application of urea on the experimental pangola grass plots was insufficient to account for the improved performance of these samples in the laboratory rumen. As stated previously, the growth stimulatory effects of foliar feeding of urea to grass apparently alters the metabolic activity within the plant to make it more subject to attack by rumen microorganisms and thus more nutritious to the host ruminant.

Urea was sprayed on a typical grass pasture in Pennsylvania, at rates of 40 and 80 lb. per acre during the month of August. The pasture, predominantly orchard and fescue grass, was mowed five weeks prior to this late summer application. As shown in table 5, the total nitrogen content of the grass from the test plots averaged 2.23 and 2.65% for urea foliar treatments of 40 and 80 lb., respectively, as opposed to 2.00% for the control. Again, the low ammonia values and the absence of residual urea were indicative of complete conversion of absorbed urea. The cellulose contents

TABLE 5. ANALYSIS OF ORCHARD-FESCUE GRASS FOLLOWING FOLIAR APPLICATION OF UREA

Item	Grass plot 1	2	3
Urea applied, lb./acre	0	40	80
Nitrogen content after[a]			
5 days, %	1.98	2.26	2.48
10 days, %	2.02	2.20	2.82
Urea content after[a]			
5 days, %	0.0	0.0	0.0
10 days, %	0.0	0.0	0.0
Ammoniacal nitrogen content after[a]			
5 days, %	0.03	0.02	0.04
10 days, %	0.02	0.01	0.01
Cellulose content after[a]			
5 days, %	27.7	28.9	27.6
10 days, %	27.9	27.4	25.2

[a] Dry matter basis.

of the samples harvested ten days after foliar-urea treatment except for the control appeared reduced when compared to those harvested after five days. This could be due in part to a dilution effect of rapid growth which ensued following the foliar feeding of urea.

Laboratory rumen data (table 6) clearly showed increased cellulolytic response of the grasses which received the foliar-urea application. This response persisted in the grass harvested 10 days after foliar treatment. The performance of the grass from the plot receiving 80 lb. of urea per acre approximated a 30% increase in the rate of cellulose digestion over the control. Similarly, in the presence of excess urea, the samples from the plot receiving 80 lb. of urea outperformed the control, even though the supplemental urea increased all cellulose digestion rates. This again

TABLE 6. *IN VITRO* PERFORMANCE OF ORCHARD-FESCUE GRASS FOLLOWING FOLIAR APPLICATION OF UREA

Item	Grass plot 1	2	3
Urea applied, lb./acre	0	40	80
Cellulose digestion of samples taken after[a]			
5 days, %	39.6	43.5*	54.7*
10 days, %	38.3	40.4	52.1*
Cellulose digestion in an excess of urea of samples taken after[a]			
5 days, %	55.3	....	62.1*
10 days, %	54.7	....	61.0*

[a] Average standard deviation ± 1.1.
* Significantly different from grass from the control plot $P < .05$.

demonstrated that the increased cellulolytic response of grass from the urea-sprayed plots is not primarily due to the increase in total nitrogen content alone.

The *in vivo* rumen evaluation (table 7) of samples harvested 10 days after foliar application confirmed the *in vitro* findings. Dry matter digestion was 56.7 and 58.9%, respectively, for urea treatments of 40 and 80 lb. to the acre as opposed to 51.5% for the control sample. Cellulose digestion, which paralleled dry matter digestion closely, was 55.2 and 58.2%, respectively, for the two rates of urea treatment in contrast to 45.8% for the control. More significant, however, were the data on protein digestibility. Foliar application of urea at 40 and 80 lb. per acre increased the digestible protein content from 3.31 to 4.82 and 7.76 lb. of protein

TABLE 7. *IN VIVO* RUMEN RESPONSE TO ORCHARD-FESCUE GRASS HARVESTED 10 DAYS FOLLOWING FOLIAR APPLICATION OF UREA

Item	Grass plot		
	1	2	3
Urea applied, lb./acre	0	40	80
Dry matter digested, %[a]	51.5	56.7	58.9
Cellulose digested, %	45.8	55.2	58.2
Crude protein digested, %	26.3	36.7	44.0
Digestible protein/100 lb. dry grass, lb.	3.31	4.82	7.76

[a] Average standard deviation ±2.1.

per 100 lb. of dry grass, respectively. In other words, digestible protein was increased 46% with 40 lb. of urea per acre and 135% with 80 lb. of urea per acre.

The data presented here point to improved nutritional quality of pasture grasses and cactus after foliar application as indicated by appreciably increased cellulose digestion *in vitro* and *in vivo*. In addition, the rapid response to foliar feeding of urea was observed. Absorption of urea was evident after eight hours in the case of the prickly pear cactus. Complete conversion of urea nitrogen by the plant and increased cellulolytic response in the laboratory rumen was evident after three days. Another feature of foliar feeding of urea is the economy involved. The data indicate that as little as 10 to 20 lb. of urea per acre gave optimum response in the pangola grass. This is in contrast with the 30 lb. or more of nitrogen per acre recommended for ground application. Finally the timing aspect of this urea application has considerable merit. Foliar application of urea can be timed with the need for increased pasture nitrogen and improved pasture quality. For example, a foliar urea spray could be timed during the summer months when pasture grasses are maturing rapidly and are showing lower protein content along with lower nutritional quality. Furthermore, foliar application of urea would be less dependent on the soil moisture content than ground application.

It is obvious from these preliminary experiments that further investigations are necessary to assess other related variables. These include (1) type of pasture, (2) stage of maturity of pasture, (3) rate of urea application, (4) time of grazing or harvest after urea application, (5) duration of urea response and (6) environmental factors, such as soil type, temperature, soil moisture, precipitation and previous history on fertilization. Of course, the final proof of these findings will await performance of the animals consuming pasture grasses, hay silages, and other forage items having received urea as a foliar spray.

As a final item, these tests have yielded a novel application for the *in vitro* and *in vivo* rumen techniques, in that it provides a means for evaluating fertilizer practices by determining the response of rumen microorganisms to the resulting pasture or forage crop.

Summary and Conclusions

Preliminary experiments indicate that foliar feeding of pasture grasses and other forage crops with solutions of urea will improve their nutritional quality. Tests with prickly pear cactus, pangola grass, and a mixture of orchard and fescue grasses which received foliar urea treatment have shown rapid uptake and conversion of urea nitrogen, increases in total nitrogen, and marked improvement in the digestibility of cellulose and protein.

The features of foliar feeding are (a) improvement in nutritional quality of plant receiving foliar treatment, (b) rapidity of response, (c) economy of application, and (d) timing of application to suit the needs of individual situations.

These preliminary tests point up the need for further research on foliar application for a fuller understanding of the variables involved. These variables include type of pasture or plant, maturity stage of plant, rate or urea application, duration of response, time of grazing or harvest after urea application and environmental factors.

The data presented indicate a novel application of the *in vitro* and *in vivo* rumen techniques for a rapid evaluation of fertilizer treatment as it affects pasture and forage quality.

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A CONCEPT OF RATE OF FOOD PASSAGE THROUGH THE GASTRO-INTESTINAL TRACT

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IN a previously reported study of coprophagy (Thacker and Brandt, 1955), rabbits were fed different diets and were either free to reconsume their feces or confined in stanchions so that coprophagy was impossible. Single feedings of radioactive chromic oxide were used as a tag for the indigestible portion of the ingesta and the radioactive chromium excreted in the feces was measured at various times after feeding. The mathematical reduction of the data from these studies has implications relative to the movement of feed residues through the gastro-intestinal tract.

Experimental

Radioactive chromic oxide was prepared from irradiated chromium (Cr-51) obtained from the Oak Ridge National Laboratory. The material as received was in the form of powdered metallic chromium. This was dissolved in HCl. (A comparable product of Cr-51 as the chloride in acid solution is now available from ORNL.) Chromic hydroxide was precipitated with ammonium hydroxide, centrifuged and washed several times to free it of chlorides. The chromic hydroxide was then dried, transferred to a silica dish and ignited in a muffle at 700° C. overnight. The resulting cake of chromic oxide was easily reduced to a fine powder. The oxide was digested for an hour in boiling 10% HCl to remove any soluble radioactive compounds. It was then washed several times with hot water, dried and crushed. This yielded a finely divided product (100 mesh) with a specific activity of approximately 35μ/mg. of oxide. With processed Cr-51 now available as the chloride, this activity could easily be doubled.

The relative concentration of the chromic oxide tag in the feces was estimated by direct counting on the dried ground feces. Half gram samples of the fecal material (40 mesh) were weighed into standard 1 in. diameter by 5/16 in. stainless-steel cups and tamped with a loose fitting steel plunger. This yielded samples of essentially the same thickness with a smooth surface. These were counted with an argon filled GM tube with a 1.4 mg./cm.^{2} window. The data were presented as counts per minute/mg. fecal material as an estimate of chromium concentration. When the quantity of feces was less than 0.5 gm. the data were corrected to the 0.5 gm. equivalent by means of an experimentally prepared curve. It was found that the overall

absorption coefficient for the radiation (the vanadium X-ray) was not significantly altered by the variations in fecal composition found in these studies. This permitted preparation of a composite sample-weight correction curve to be used for all samples as necessary. The reproducibility of the method is excellent; sampling, packing and counting represent a standard deviation of less than 3%.

Other experimental details have been described (Thacker and Brandt, 1955).

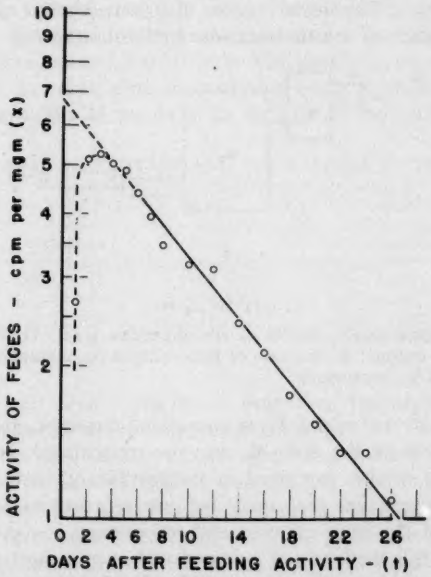


Figure 1. Excretion of $Cr_2^{51}O_3$ in 2-hour periods by a rabbit fed a purified diet. Feces collected in free state.

Results

Using the counting rate of the feces, expressed as counts/min./mg. fecal material, as an estimate of chromium concentration at time t after feeding, all our data fit the general relation,

$$x = Xe^{-kt} \text{ for } t > n \qquad (1)$$

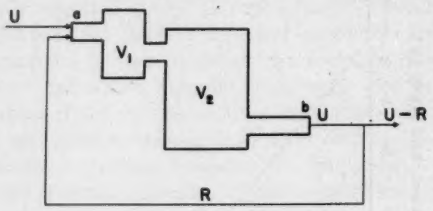
X is a constant depending on the level of active chromium fed. The parameter k depends on the diet and state of confinement. The expression as given holds only after the time n has elapsed. A plot of representative data is given in figure 1.

From this figure, note that there is a short delay in which no activity is excreted, then a very rapid rise to a maximum and then an exponential relation as defined in equation (1). The delay area, depending on the sensi-

tivity of the technique, represents what is generally called "transit time". The exponential portion of the curve is important from the standpoint of the actual time feed residues are subject to digestive processes.

Discussion

In order to try to evaluate the degree of recirculation, it is necessary to express k, the exponential slope in (1), in terms of flow rates and recirculation rates. The model shown in figure 2 based on a hydraulic analogy was chosen. This model consists of a delay pipeline and two volumes V_1 and V_2 in each of which complete and instantaneous mixing occurs.



$x = C(e^{-k_1 t} - e^{-k_2 t})$

Figure 2. Diagrammatic model of the digestive tract. U, the rate of feed intake and fecal output; R, the rate of fecal output reconsumed; a and b delay pipelines; V_1 and V_2, reservoirs.

The flow through the model, U, is considered constant, and input equals output. A portion of the flow, R, may be recirculated. In terms of the rabbit, the feed intake, expressed in indigestible or fecal units, and the collected fecal excretion are equal and are represented by the rate U. The quantity of the feces excreted and reconsumed is represented by the rate R. If into this model a tag is introduced over a short period of time, the concentration of the tag, X, appearing in the output at time t after feeding will be given by

$$x = C(e^{-k1(t-m)} - e^{-k2(t-m)}) \quad t \geq m \qquad (2)$$
$$x = 0 \quad t \leq m$$

This equation is of the form of figure 1 and after the time m reduces to the form of equation 1.

Unfortunately, the form of the coefficients, k, in (2) are rather complicated functions of U, V, and R, which preclude its use for estimating R. In the case of the rabbit with the very rapid initial rise in the concentration of tag vs. time, one of the volumes can be considered negligible. Treatment of a single volume model gives rise to an equation identical to (1) with the added restriction

$$x = 0, \; t < n \qquad (1a)$$

Using this form, some estimate of R can be obtained.

The important point, however, is that this model will reproduce the experimental curves of the rabbit, that one of the volumes is negligible and

that a single parameter k describes the curve adequately. Because the exponential portion of the curve is important from the standpoint of the actual time feed residues are subject to digestive processes, it is suggested that a derived "half time" is an appropriate, justifiable and descriptive parameter by which to describe feed passage through the rabbit.

In table 1, the effects of diet and coprophagy on the calculated half times of feed residue in the rabbit are presented for three different diets. The half times are derived from the k obtained by a least squares fit of relation 1 to the experimental data and are numerically equal to 0.693/k. Transit time as measured by the first appearance of the tag or by the time the maximum was reached, varied little with diet or state of confinement. The half times, however, offer a reasonable explanation of observed differences in digestibility of the diets. In the case of the purified diet in the

TABLE 1. FECAL EXCRETION AND HALF TIMES IN THE RABBIT

Diet	Total feces (gm./day)		Half times (days)	
	Stanchioned	Free	Stanchioned	Free
Milk	2.54(0.779) [a]	2.13(0.288) [a]	5.8 (1.20)	13.1 (2.15)
Purified	5.24(0.475)	3.55(0.596)	1.3 (0.13)	8.7 (1.17)
Roughage	17.7 (1.02)	14.1 (1.02)	0.35(0.026)	0.92(0.071)

[a] Standard error.

free state, the half time is about six times and the digestibility of the cellulose two times that in the stanchioned state.

To indicate the applicability of this model and method of treatment to another species the data of Balch *et al.* (1954) with cows is pertinent. Balch used stained feed and counted the number of stained particles appearing in the feces as a function of time. He presented his data as the accumulation of excreted particles rather than as a concentration. Using his data and by assuming a constant fecal excretion, the differential curves with concentration vs. time may be obtained. These curves are of the form of relation (2). On the basis of the model, both volumes are significant. It is possible to resolve the derived curves into two exponential components and obtain two half times derived from k_1 and k_2. These half times are presented in table 2.

Balch (1950) in a rather extensive study of feed passage through the cow used a 5% excretion time and an 80 minus 5% excretion time as descriptive of his accumulation curves. These are, the times required for excretion of 5% of the stained residues and the difference between the times at which 80 and 5% of the residues are excreted. From the data obtained by fistulated animals he proposed that the 5% excretion was indicative of the time taken by undigested residues to pass through from the omasum, abomasum and intestines. Further he proposed that the 80 minus 5% excretion time indicated the time residues remained in the reticulo-rumen. These measures of the curves are also presented in table 2.

The measures used by Balch to describe his curves have no mathematical basis. If the form of his curves is the integral of (2), as they appear to be, the description of the data by two half times is warranted. It is, however, an unfortunate characteristic of this relation that one cannot assign a flow order to the exponential coefficients without an independent indication of the sign of C. Balch's work would indicate that the longest half time (smallest k) would be in the first portion of the tract. Since C in (2) contains the sign determining the expression $\frac{1}{k_2-k_1}$ this would indicate that C was positive.

In the derived half times presented in table 2, it has been arbitrarily assumed that C was positive. In considering the digestive tract of the ruminant and assuming that it behaves in a manner as the proposed model, one is lead intuitively to this same conclusion.

TABLE 2. HALF TIMES IN THE RUMINANT

(From C. C. Balch *et al.* J. Dairy Res. 21:313, 1954)

Diet	Tag excretion times (hr.)		Half times (hr.)	
	5%	80–5%	T_1[a]	T_2[a]
I	27.6	66.6	11	28
II	28.8	120.6	10	64
III	33.0	130.4	19	24

[a] Calculated from relations (2) assuming C is positive. $T_1=0.693/k_1$. T_2 and T_1 presumptively refer respectively to the reticulo-rumen portion and to the omasum, abomasum, intestine portion of the tract.

It must be kept in mind that in both the rabbit and ruminant, the model presented is simply a convenience in handling the experimental data obtained in such studies. The passage of feed residue through the animal is such that the digestive tract appears to behave in the manner of the model. This does not mean that the volumes of the model exist as physical units in the tract but only that the entire tract behaves as though it were two volumes insofar as feed residue passage is concerned.

After this paper was prepared, the work of Blaxter *et al.* (1956) was called to our attention. These authors, working with sheep and following the procedures of Balch, used a kinetic model with two successive rate constants and arrived at an equation identical in form and subject to the same limitations as our equation 2. Then, with this single-meal flow relation and applying the superposition theorem of Heaviside and the Dirac-function, they derived a relation for the diurnal variation in the rate of feces production. The use of this relation to calculate the residual effect of a previous ration on the fecal output is of value in determining the number of days required on a new ration to obtain a practicable steady state.

In a discussion of digestibility, these authors introduced the concept of mean-time as a description of the passage of a single meal. Since they used the stain-particle technique, this time was defined as the sum of the times which individual stained particles spent in the tract, divided by the total number of particles. It was not shown that this time could be calculated from the parameters of their flow equation. While this could be done, it is not felt that this concept is any easy one to use. Certainly, the single value does not adequately describe the shape of the flow curve for the ruminant. The expression of the parameters as half times is a concept which is easier to grasp. The important point is that the passage of feed residues is an exponential function and subject to mathematical treatment.

Mäkelä (1956) developed a concept of a "food dry matter point" which is defined as the average time, in days, of retention of a dry matter point in the gastro-intestinal tract or its parts. When expressed in terms of a food lignin point, this concept is comparable to Blaxter's mean-time.

The relative simplicity of the radiochromium technique, the low levels of radioactivity required, the short half-life of the isotope and the indigestibility of the chromic oxide permits its use as an adjunct to routine digestibility studies. The observation that the passage of feed residues through the digestive tract is an exponential function, not a simple pipe line flow and the development of the concept half-time to describe this physiological phenomenon should be useful in evaluating the influence of "digestion time" on the digestibility of ration components.

Summary

Data are presented which indicate that flow of ingesta through the animal follows an exponential relation. A model based on a hydraulic flow through two volumes in which complete mixing takes place is proposed. The data indicate that with the rabbit a single volume model is adequate and that a single half time is sufficient to describe the flow of feed residues. Data from previously published work by Balch are presented to support the premise that in the ruminant a two volume model describes the observed data and that two half times are sufficient to describe the flow of feed residues in the ruminant.

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SERUM TRANSAMINASE IN WHITE MUSCLE DISEASE [1, 2]

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THE increased serum glutamic-oxalacetic transaminase (SGO-T) associated with tissue destruction has received considerable recent attention. SGO-T is responsible for the transfer of the alpha-amino group of aspartic acid to alpha-ketoglutaric acid. It is normally present in tissue and is released to the blood as a result of the breakdown of the cell barrier such as occurs in necrotic tissue.

Recently White and Hess (1957) demonstrated an increase in SGO-T in human progressive muscular dystrophy. This increase was of the order of 300% in children and 130% in adults. Merrill *et al.* (1956) reported increased SGO-T in man in two cases of skeletal muscle necrosis, in necrosis of abdominal viscera and infarctions of the liver. Increased SGO-T in myocardial infarction has received considerable attention (Merrill *et al.*, 1956; Kattus *et al.*, 1956; and LaDue *et al.*, 1954).

The purpose of this study was to determine if the SGO-T increased in white muscle disease (muscular dystrophy) in lambs and calves.

Methods

SGO-T was determined in the blood serum of normal lambs and calves and animals suffering pathological changes one to two months of age. White muscle disease was diagnosed only at autopsy rather than by subjective inspection. The pathological cases were spontaneous and purchased from various ranches in Western Nevada. The control blood samples were obtained from University of Nevada animals.

SGO-T was determined by the method of Cabaud *et al.* (1956) which was based on the method of Tonhazy *et al.* (1950). The blood serum is incubated 20 minutes at 25° C. with phosphate buffered (pH 7.4) aspartic-ketoglutaric acid substrate. The oxalacetic acid resulting from the deamination of aspartic acid is reduced to pyruvic acid which is reacted with 2,4-dinitrophenylhydrazine. The resulting hydrazone is extracted with toluene and the brown color developed with alcoholic potassium hydroxide, which is measured colorimetrically (4900 Å).

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[2] A report of this work was presented at the annual meeting of the Western Section of the American Society of Animal Production, July 14–17, 1957.

[3] Department of Agricultural Chemistry.

Enzymatic activity is expressed as micrograms pyruvic acid liberated per milliliter of serum in 20 minutes at 25° C. (SGO-T units per ml.)

Results and Discussion

Statistical study of duplicate analyses (Youden, 1951) indicated the standard error of analysis to be ±6.6% for one analyst and ±13.0% for another. It was also found that the SGO-T activity is not affected by allowing the blood to clot overnight at room temperature before centrifugation and removal of the serum.

Table 1 summarizes the data obtained. The only pathological cases included are those in which white muscle disease was verified by autopsy. The control sheep were from two University of Nevada flocks, presumably normal, in which no white muscle disease had occurred. The control calves

TABLE 1. SERUM GLUTAMIC-OXALACETIC TRANSAMINASE IN CONTROL AND PATHOLOGICAL ANIMALS

	White muscle		Normal		
	Lambs	Calves	Lambs		Calves
Mean, unit/ml.	1890	1313	128	56	57
Standard error	±254	....	±18	±31	±17
Range, unit/ml.	687–3460	295–2360	97–191	22–160	19–99
Number	17	4	20	21	69

were from a University of Nevada herd and a private herd in which no white muscle disease had occurred.

A large increase in SGO-T occurred in animals with white muscle disease. The increase was more pronounced in lambs than in cattle. All lambs studied had extensive white muscle lesions of the skeletal muscles. The four calves studied had heart lesions of varying degrees and one animal also had lesions of the skeletal muscles. In order of the severity of the heart lesions, the SGO-T activity were 295, 890, and 1670 units per ml. The calf with both skeletal and cardiac lesions had a SGO-T activity of 2360 units per ml. This may indicate that the activity of SGO-T is proportional to the extent of the lesions as was shown for experimental myocardial infarctions by Agress *et al.* (1955).

There was good agreement between the SGO-T activity of the two groups of control calves studied (57 and 58 units per ml.) and the data were combined in table 1. There was, however, a statistically significant difference between the two control groups of lambs (56 and 128 units per ml.). Inasmuch as white muscle disease had not occurred in either group, the cause of this difference is obscure but under study. In any event, the difference between the two control groups is vanishingly small when compared to the difference between the control and group showing pathology.

The increased SGO-T found in white muscle disease demonstrates the breakdown of the cell-blood barrier permitting this enzyme to enter the blood. It would appear that this increased SGO-T might be used to aid the objective diagnosis of white muscle disease, and to follow the course of this disease and its therapy. SGO-T is not increased by scours and only slightly increased by staphylococcus meningitis (132 units per ml., one case), toxemia (42 and 188 units per ml.), and severe internal injuries (82 units per ml.). While SGO-T would be increased in any condition resulting in necrotic tissue, it would appear that white muscle disease would be the most probable condition causing both stiffness *and* increased SGO-T. Herd surveys using this method are currently in progress.

Summary

Serum glutamic-oxalacetic transaminase activity increased markedly in lambs and calves with white muscle disease (muscular dystrophy). The serum transaminase activity appears to be proportional to the extent of muscle damage. This increase may permit the objective diagnosis of white muscle disease without sacrifice of the animal.

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A TECHNIQUE FOR ESTIMATION OF THE EFFECTIVENESS OF GOITROGENS IN CATTLE [1, 2]

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THE role of the thyroid gland and its hormones, thyroxine and triiodothyronine, in stimulating growth, reproduction, lactation, egg production and the skin appendages such as feathers, hair, fur and wool have been studied. These studies suggest that the secretion of optimal levels of these hormones is a prerequisite for superior reproductive processes in farm animals. On the other hand, in the rapid fattening of farm animals, a hypothyroid condition is highly desirable for most economic gains (Winchester and Andrews, 1953; Hill, 1953).

As pointed out by Blaxter *et al.* (1949), in the case of the thyroid gland, it is now possible to produce varying degrees of hyperthyroidism simply by feeding thyroprotein and varying degrees of hypothyroidism by feeding goitrogens which block thyroxine secretion.

Considerable study has been given to the use of goitrogens in partially or completely blocking thyroxine secretion in order to promote the fattening of poultry and swine (Blaxter *et al.*, 1949; Hill, 1953; Winchester and Andrews, 1953). However, the use of these compounds in ruminant animals, such as cattle and sheep, has been very limited because of a lack of knowledge of the amount of goitrogens required to control thyroxine secretion in these animals effectively, (Andrews *et al.*, 1947; Beeson *et al.*, 1947).

The dramatic effect of a hypothyroid state in the rapid fattening of cattle was described by Bullard and Andrews (1943) when beef animals were partially thyroidectomized. However, in these animals subsequent thyroid hypertrophy soon restored thyroid hormone secretion to normal with a rapid lessening of the fattening rate. A state of hypothyroidism for a period following the rapid withdrawal of thyroprotein has been observed in dairy cattle. During this period, very rapid gains in weight have been reported. While these rapid gains in weight have been ascribed to fill, increased water content of the tissue, etc., it has been shown by Swanson (1954) not to be due to increased body water. The writers consider that increased body weight gains are an indication of more rapid fattening associated with lowered metabolic rate.

In recent years, the favorable effect of estrogens upon the rate and efficiency of gains has been observed in cattle and sheep. While these growth gains have been impressive, the carcass quality has been reported to suffer indicating a stimulus of growth at the expense of fattening. It

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has been suggested that in estrogen stimulated animals, the rate of fattening and carcass quality of the finished animal could be improved by the use of goitrogens toward the end of the feeding period.

The most common method of determining the optimum level of thiouracil administration has been the determination of the increase in the size of the thyroid gland resulting from the blockage of thyroxine secretion and the following hyperplasia caused by increased secretion of thyrotropin. Since data of this type can be obtained only upon the sacrifice of the animal, this method cannot be applied to cattle except at considerable cost.

Ely *et al.* (1948) studied the thiouracil blood plasma level of goats and calves in relation to dosage in order to attempt to estimate minimum effective levels for thyroid blockage.

Stanley and Astwood (1947) described a method based upon reduced rate of radioiodine uptake by the thyroid gland when goitrogens were administered. While this method is satisfactory for qualitative comparisons of goitrogens, its sensitivity is limited due to normal variations in uptake of radioiodine.

Goitrogens exert their effect by preventing the formation of organic iodine (thyroglobulin) in the thyroid gland (Vanderlaan and Bisell, 1946). Under these conditions the thyroid cannot retain inorganic iodine and the net result is a rapid reduction in the preformed thyroid hormone content of the gland.

The object of the present report is to describe an improved method of determining the amount of thiouracil or methyl-thiouracil required to block uptake of iodine and the further secretion of thyroxine by thyroid glands of cattle (Pipes and Turner, 1956).

Experimental Procedure

Cattle were injected intravenously with carrier-free NaI^{131} and measurements of thyroidal I^{131} were made at 24-hour intervals by the method described by Pipes *et al.*, 1957. Goitrogens were administered by gelatin capsule at 24-hour intervals. The animals were allowed free access to iodized salt. Results were expressed as percent of injected dose after correction for radioactive decay.

Experiment I. Four non-lacting heifers were injected with radioiodine and measurements of thyroidal I^{131} were made during a control period of four days to establish the normal rate at which I^{131}-tagged thyroxine was leaving the thyroid gland. It is complicated by the fact that I^{131} from metabolized thyroxine also returns to the thyroid gland and is utilized a second time for the formation of I^{131}-thyroxine. This phenomenon has been called "recycling" or "reutilization" of iodine.

If a goitrogen is now administered in sufficient amount to prevent the uptake of the recycled I^{131} and synthesis of additional thyroxine by the thyroid gland, there would be an increase in the apparent rate of thyroxine

discharge from the thyroid gland, since the goitrogens have no effect upon the release of thyroxine already present in the gland but block I^{131} uptake.

When 6-methyl-thiouracil was administered at the rate of 1 gm. per 100 lb. body weight for 6 days, measurement of thyroidal I^{131} 24 hours later showed an increase in the rate of release of thyroxine-I^{131} indicating that the goitrogen acts swiftly in blocking iodine uptake. Measurements

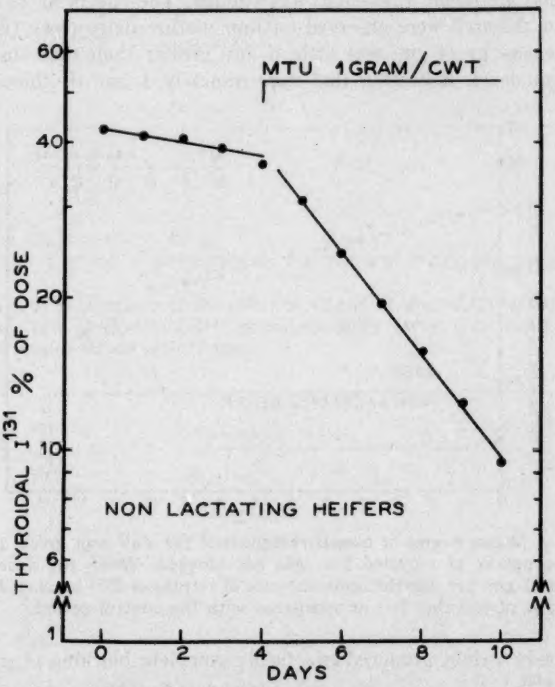

Figure 1. The average rate of release of thyroidal-I^{131} in four non-lactating heifers is shown during a 4 day control period. Upon the administration of 1 gm. of 6-methyl-thiouracil (MTU) per 100 lb. body weight per day, the uptake of recycled I^{131} was prevented and a sharp break in the slope resulted.

made during the next five days indicate an exponential decrease of thyroidal I^{131}. During this period of time the rate of release did not vary, indicating that sufficient hormone was present in the gland to meet daily requirements (figure 1).

In order to test the effect of smaller dosages, one Guernsey heifer weighing about 1100 lb. was given 6-methyl-thiouracil for two days at the rate of 6 gm. per day after control period of four days. Little or no effect on the release rate of thyroidal I^{131} was observed. Therefore, the dose was

increased to 12 gm. per day (approximately one gram per 100 lb. body weight). At this dosage a prompt effect upon the release rate was observed (figure 2).

From these observations, it was concluded that blockage of uptake of I^{131} by the thyroid gland was effected by the daily administration of 1 gm. of 6-methyl-thiourcil per 100 lb. body weight.

A second goitrogen, thiouracil, was studied. The effects of 12 gm. and 24 gm. of thiourcil were observed on four mature dairy cows (figure 3). The responses to 24 gm. was little if any greater than that observed at the 12 gm. level, indicating that approximately 1 gm. of thiouracil per

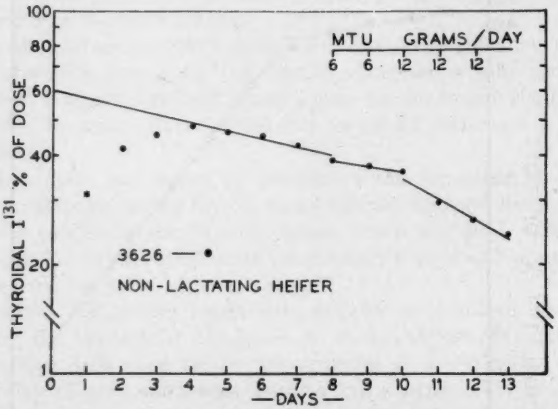

Figure 2. When 6 gm. of 6-methyl-thiouracil per day was given a 1100 lb. heifer, the uptake of recycled I^{131} was not blocked. When the dose was increased to 12 gm. per day the apparent rate of release of I^{131} increased, indicating blockage of recycled I^{131} as compared with the control period.

100 lb. body weight produced practically complete blocking of uptake of recycled I^{131}.

Mature dwarf beef animals weighing approximately 600 lb. were found to have as great a response to 6 gm. of thiouracil as to 12 gm. (figure 4). These results confirmed previous findings that 1 gm. per 100 lb. body weight produced maximum effect.

Experiment II. In these studies on young dairy animals thiouracil was administered at three different dose levels, each in a separate trial. In order to make comparisons between trials, the rate constant for the net release rate of thyroid hormone (k'4) was computed according to the method of Brownell (1951). The higher the k'4 value the more complete is the inhibition of the thyroid's ability to collect iodine. The net result is a more rapid apparent loss of iodine from the thyroid, since iodine is being collected at a slower rate or not at all.

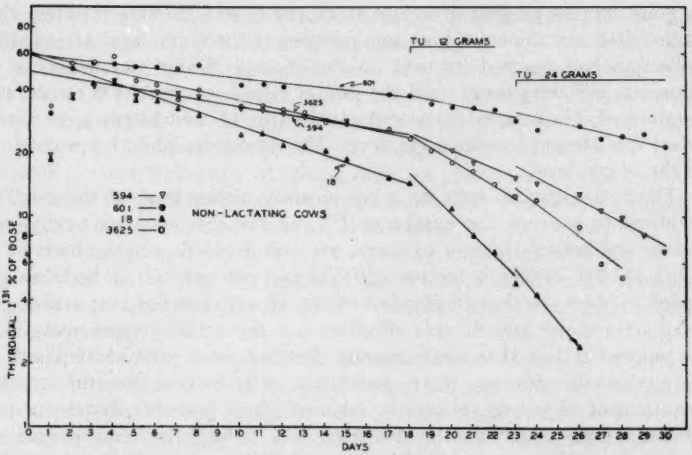

Figure 3. A comparison of the effect of 12 and 24 gm. of thiouracil upon the net release rate of thyroidal I^{131} in mature dairy cattle. Practically complete inhibition was observed with 12 gm.

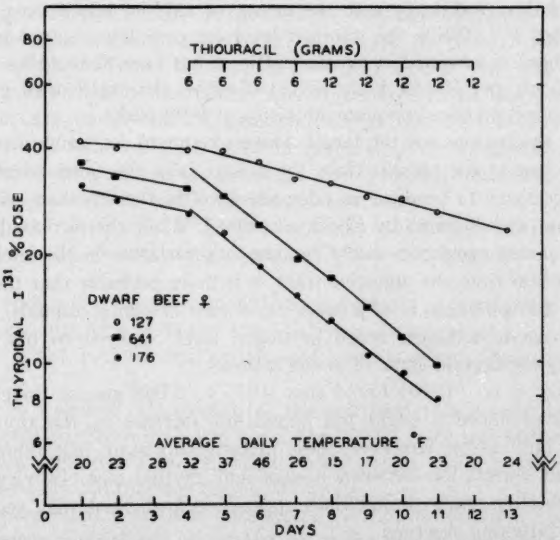
THIOURACIL (GRAMS)
6 6 6 6 12 12 12 12 12
DWARF BEEF ♀
127
641
176
THYROIDAL I 131 % DOSE
AVERAGE DAILY TEMPERATURE °F
20 23 26 32 37 46 26 15 17 20 23 20 24
DAYS

Figure 4. The effect of 6 gm. and 12 gm. of thiouracil upon the apparent release rate of thyroidal I^{131} in dwarf beef animals after a control period of 3 days. Maximum inhibition was found at the 6 gm. level or 1 gm. per 100 lb. body weight.

Four animals ranging in weight from 320 to 415 lb. were injected with radioiodine and thiouracil was administered at the 6 gm. level after iodine collection had reached its peak in the thyroid. Daily measurements of thyroidal I^{131} were made until the rate of release of thyroid hormone was established. The experiment was repeated at the 12- and 24-gm. level. Little effect was observed at the 6 gm. level. The maximum inhibition was found at the 24 gm. level.

These observations indicate a significantly higher level of thiouracil is required to prevent the uptake of I^{131} by the thyroid glands of young calves and heifers. Instead of 1 gm. per 100 lb. body weight observed to block the I^{131} uptake in mature cattle, 3 to 4 gm. per 100 lb. body weight failed to block the thyroid gland of calves. It was observed that 6 to 8 gm. of thiouracil per 100 lb. was effective, but the actual requirements may be somewhat less than that amount. Further work will be required to determine the amount more accurately. The reason for the greater requirement of young animals is unknown. It is probable that more frequent administration of the goitrogen will reduce the requirements to some extent, due to the rapid elimination of the chemical (Ely *et al.*, 1948).

Discussion

Unlike methods based on increase in thyroid size, our results relate thiouracil dosage directly with the degree of thyroid inhibition of uptake of recycled I^{131}. While the minimal level for complete thyroid inhibition has not been determined in mature cattle, it has been shown that approximately 1 gm. per 100 lb. body weight of either thiouracil or of 6-methylthiouracil will produce complete inhibition of I^{131} uptake.

While the reasons for the larger dosages required for immature animals are not clear at the present time, the dosage is in the same range as that found necessary to produce an adequate level in the blood of calves (0.1 to 0.2 gm. per kilogram) by Ely *et al.* (1948). While the increased requirement of young ruminants might be due to a variation in the effectiveness of absorption from the digestive tract, it is more probable that the metabolism of the goitrogen is at a more rapid rate in young animals. It could also be due to a higher blood goitrogen level required to block iodine uptake by the thyroid gland of young animals.

Schultze *et al.* (1950) found that 0.035 to 0.065 gm. of thiouracil per kilogram produced a slight but significant increase in the thyroid size of dairy bull calves. However, these investigators state that there was no significant correlation between dosage and thyroid size. In view of the present findings, these dosage rates appear too low, which may account for the poor fattening observed.

Since the work of Pipes and Turner (1948) and that of Ely *et al.* (1948) showed a rapid rate of disappearance of thiouracil from the blood, smaller

dosages, perhaps, could have been used in the present study, if the dose were divided and administered at 12 hour intervals.

In the present investigations the rate of release (figures 1 and 4) is unchanged for 8 days or longer, under constant thiouracil administration. These observations are interpreted as indicating that the thyroid stores of preformed hormone is sufficient to supply body requirements for a number of days. Since rate of release does not change, evidently the level of hormone in the blood does not drop during this period and increased amounts of thyrotrophic hormone are not secreted.

In the use of goitrogens in cattle feeding experiments it should be pointed out that a hypothyroid condition is not produced until the store of preformed thyroxine is utilized. In mature cattle this may require about 2 weeks.

These data on cattle indicate the dosage of either thiouracil or methyl-thiouracil which could be fed to cattle toward the end of the feeding period to increase the rate of fattening and carcass quality. In practice, the amount of goitrogen may be reduced below the level indicated because it would be undesirable to completely block thyroxine secretion.

Summary and Conclusions

A new technique is described for the estimation of the biological activity of goitrogens in intact cattle. It involves the administration of I^{131} and the subsequent measurement of the rate of release of thyroxine-I^{131} from the thyroid gland. When the goitrogen to be tested is then fed, there is a break in the slope of the release rate curve due to the blocking of the further uptake of I^{131} which otherwise occurs due to the recycling or reutilization of iodine coming from the metabolism of thyroxine.

The two goitrogens tested, thiouracil and 6-methyl-thiouracil, were of equal biological activity within the range of the tests.

The uptake of recycled I^{131} was completely inhibited by the once daily feeding of 1 gm. of either goitrogen per 100 lb. body weight in mature cattle.

It was shown that the performed thyroxine present in the gland is released at a uniform rate for a period of 8 to 14 days, indicating that a hypothyroid condition is not induced until the thyroid gland is essentially exhausted of thyroxine.

In calves weighing 300 to 400 lb., the goitrogen requirement is considerably increased. More than 3 to 4 gm. per 100 lb. body weight is required. It was shown that 6 to 8 gm. per 100 lb. body weight would produce greater inhibition of thyroid function than lower dosages.

It was suggested that these data would provide information concerning the goitrogen requirement of cattle toward the end of the feeding period to increase the fattening rate and to increase the carcass quality of cattle by the induction of a degree of hypothyroidism.

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MULTIPLE BIRTH IN THE TEXEL SHEEP

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IN August 1955, the first importation of Texel sheep from Holland arrived at the Tahreer Province in Egypt. It consisted of 25 ewes and 2 rams. Afterwards, it was followed by a group of 121 ewes and 2 rams in November, and finally by 239 ewes and 6 rams in December of the same year, making a total number of 385 ewes and 10 rams. The animals came from about 40 different flocks scattered all over Holland.

As the imported animals represent, to a large extent, a random sample of the breed, their pedigree records were studied to gain some information on the merits of the Texel sheep in its homeland. The number of lambs born, lambs reared up to weaning age, and heritability of multiple birth were studied and discussed. Such information will be useful along with that concerning the adaptation of this breed in Egypt, as soon as it becomes available.

Material and Method

The data used in this study were obtained from the dams, dams' dams, and sires' dams of the imported sheep. The number of ewes in the three mentioned groups were 333, 275 and 146, respectively. The number in each group is less than the number of animals imported, because some of the ewes were dams to more than one animal while some rams were sires to many of these ewes and also some animals had incomplete pedigrees.

Table 1 presents the data available in the three groups of ewes distributed according to their year of birth. Almost 76% of the dams were born in the years from 1950 to 1953, 69% of the dams' dams in the years 1946 to 1950 and 69% of the sires' dams in the years 1948 to 1951. The sires' dams can be considered as being from the dams' dams generation, although they were not quite as old. This may be due to the fact that dams stay in the flock longer than sires.

The distribution of ewes according to their month of birth is given in table 2. Some ewes did not have their month of birth identified. The majority (93.3%) were born between the first of March and the middle of April. Only 4.4% were born in the second half of February and 2.3% in the second half of April.

No attempt was made to separate the ewes according to flocks because the number within each flock were small, and they varied considerably.

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TABLE 1. DISTRIBUTION OF DAMS (D), DAMS' DAMS (DD), AND SIRES' DAMS (SD) ACCORDING TO YEAR OF BIRTH

Year	Number			Year	Number		
	D	DD	SD		D	DD	SD
1939	..	2	..	1949	32	42	24
1941	..	6	..	1950	52	40	22
1942	..	6	2	1951	63	28	28
1943	1	16	..	1952	79	4	13
1944	1	20	6	1953	70	2	4
1945	2	16	4	1954	6	..	..
1946	1	45	9	1955	1	..	..
1947	19	51	10				
1948	22	45	33	Total	349	323	155

Results and Discussion

Tables 3, 4 and 5 present means, variances, standard deviation and coefficients of variability for number of lambs born, and number reared for dams, dams' dams and sires' dams, respectively. Analysis of variance was carried out to test the differences between the three groups of ewes in the different number of lambings. Differences were not statistically significant except for the class of six lambings for the reared lambs where they were highly significant. Data in tables 3, 4 and 5 were pooled in table 6.

Number of Lambs Born. The average number of lambs born per lambing was 1.91 based on an average of 3.4 births per ewe. This high average may have resulted in part from selection because ewes having more lambs might be more apt to have one available for sale. Smirnov (1935) reported that the Romanov sheep in U.S.S.R. produce 5% singles and 90% twins and triplets. Johansson and Hansson (1943) gave an average of 1.50 for the Landrace, Oxford, Cheviot and Shropshire sheep. Desai and Winters (1951a) estimated the overall average of several lines of purebred and crossbred sheep as 1.44. Terrill (1952) reported an estimate of 1.23 for

TABLE 2. DISTRIBUTION OF DAMS, DAMS' DAMS, AND SIRES' DAMS ACCORDING TO MONTH OF BIRTH

Groups of ewes	Feb.	March		April	
	16–29	1–15	16–31	1–15	16–30
Dams	10	119	141	52	7
Dams' dams	14	88	128	77	9
Sires' dams	11	48	67	22	2
Totals	35	255	336	151	18
Percent of all ewes	4.4	32.0	42.3	19.0	2.3

TABLE 3. MEANS, VARIANCES, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIABILITY FOR NUMBER OF LAMBS BORN AND NUMBER REARED FOR DAMS

Number of lambings	1	2	3	4	5	6
No. of ewes	74	88	69	50	31	21
No. of lambs born	143	321	414	370	300	240
Mean	1.93	3.65	6.00	7.40	9.68	11.43
Variance	.201	.461	1.147	1.020	1.892	1.157
Standard deviation	.448	.679	1.071	1.010	1.376	1.076
Coefficient of variation	23.2	18.6	17.8	13.6	14.2	9.4
No. of lambs reared	132	283	340	320	261	201
Mean	1.78	3.22	4.93	6.40	8.42	9.57
Variance	.227	.608	1.362	1.837	2.252	2.107
Standard deviation	.477	.780	1.167	1.355	1.501	1.450
Coefficient of variation	26.8	24.2	23.7	21.2	17.8	15.2

the Rambouillet. In the Egyptian Sheep, Ragab and Asker (1954) found that the average was 1.20 in the Ossimi while Karam (1956) found that it was 1.24 in the Rahmani.

Table 6 presents the average number of lambs born for ewes having had 1, 2, 3, 4, 5, and 6 lambs. They were 1.91, 3.66, 5.90, 7.37, 9.67 and 11.76, respectively. Since the number of lambs per birth for each ewe was not recorded, the approximate average number of lambs per birth at the different lambings was obtained by subtraction using the averages given in table 6. The result was 1.91, 1.75, 2.24, 1.47, 2.30 and 2.09 for the first up to the sixth lambing. The number of lambs per birth increases with the advance of age of the ewe with the maximum being reached between 5 to 8 years of age depending on the breed (Johansson and Hansson, 1943;

TABLE 4. MEANS, VARIANCES, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIABILITY FOR NUMBER OF LAMBS BORN AND NUMBER REARED FOR DAMS' DAMS

Number of lambings	1	2	3	4	5	6
No. of ewes	14	25	60	70	64	42
No. of lambs born	26	89	346	517	605	498
Mean	1.86	3.56	5.77	7.39	9.45	11.86
Variance	.285	1.007	1.199	1.545	1.998	3.686
Standard deviation	.535	1.003	1.095	1.243	1.413	1.920
Coefficient of variation	28.8	28.2	19.0	16.8	15.2	16.2
No. of lambs reared	24	79	307	458	517	442
Mean	1.71	3.16	5.12	6.54	8.08	10.52
Variance	.225	.877	1.393	1.440	2.073	3.182
Standard deviation	.475	.936	1.180	1.200	1.440	1.784
Coefficient of variation	27.8	29.6	23.0	18.3	17.8	17.0

TABLE 5. MEANS, VARIANCES, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIABILITY FOR NUMBER OF LAMBS BORN AND NUMBER REARED FOR SIRES' DAMS

Number of lambings	1	2	3	4	5	6
No. of ewes	9	27	32	36	22	20
No. of lambs born	16	102	191	263	226	238
Mean	1.78	3.78	5.97	7.31	10.27	11.90
Variance	.195	.493	.732	1.304	3.922	.726
Standard deviation	.442	.702	.856	1.142	1.981	.852
Coefficient of variation	24.8	18.6	14.3	15.6	19.3	7.2
No. of lambs reared	16	83	165	237	187	227
Mean	1.78	3.44	5.16	6.58	8.50	11.35
Variance	.195	.996	.846	1.907	2.262	1.503
Standard deviation	.442	.999	.919	1.381	1.504	1.226
Coefficient of variation	24.8	29.0	17.8	21.0	17.7	10.8

Desai and Winters, 1951a; Ragab and Asker, 1954; Rendel, 1956; and Karam, 1956). This trend was not clear in the present study because the effect of year and the effect of the age of the ewe were partially confounded. For example, more than half of the dams with one lambing and also more than half of those with two lambings were born in the years 1953 and 1952, respectively. Also, selection of ewes which enter into averages at older ages, participates in increasing the averages. Nevertheless, it can be concluded that the maximum number of lambs per birth was reached at the fifth lambing.

Number of Lambs Reared. The average number of lambs reared per lambing was 1.67, for all the ewes. This average may be high for the same reason given for the number of lambs born. The average number of lambs reared by ewes with 1, 2, 3, 4, 5 and 6 lambings were 1.77, 3.18, 5.04, 6.44, 8.40 and 10.48, respectively (table 6). When the number of

TABLE 6. MEANS, VARIANCES, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIABILITY FOR NUMBER OF LAMBS BORN AND NUMBER REARED FOR ALL EWES

Number of lambings	1	2	3	4	5	6
No. of ewes	97	140	161	156	117	83
No. of lambs born	185	512	951	1150	1131	976
Mean	1.91	3.66	5.90	7.37	9.67	11.76
Variance	.210	.558	1.078	1.306	2.379	2.331
Standard deviation	.459	.747	1.038	1.143	1.543	1.527
Coefficient of variation	24.0	20.4	17.6	15.5	15.9	13.0
No. of lambs reared	172	445	812	1015	965	870
Mean	1.77	3.18	5.04	6.44	8.40	10.48
Variance	.219	.724	1.267	1.658	2.154	2.862
Standard deviation	.468	.851	1.126	1.288	1.467	1.692
Coefficient of variation	26.4	26.8	22.3	20.0	17.5	16.1

lambs reared per birth at the different lambings was calculated no obvious trend was noted but the maximum number was at the sixth lambing.

Mortality Rate. The difference between the number of lambs born and the number reared represents the mortalities among lambs from birth up to weaning age. For ewes with 1, 2, 3, 4, 5 and 6 lambings the mortality percentages were 7.03, 13.09, 14.62, 11.74, 14.68 and 10.86, respectively. Differences between them were not statistically significant. Their average mortality rate based on all lambings was 13%. The mortality rate for ewes with one lambing would obviously be biased downward because ewes having lambs born but failing to rear any would be automatically excluded. This would probably also be true of the last year for each group but the bias would become less important as the number of lambings increase. This may help account for the generally low mortalities found. Selection may enter in also as ewes with a high mortality of their lambs would be less apt to have offspring available for sale. Venkatachalam *et al.* (1949) gave an estimate of 28.6% for the average mortality rate from birth up to 2 months of age in several breeds of sheep. The different percentages ranged from 18.0 to 42.2. An estimate of 18.8% was calculated from Terrill's data (1952) for Rambouillet lambs at weaning age. He also reported that ewes at two years of age had the highest lamb mortalities. Ragab *et al.* (1954) reported that mortality rates until weaning age in the Ossimi and Rahmani sheep were 11.5 and 9.3%, respectively. In the Rahmani flock of the Alexandria University the mortality rate, at two months of age, was 18.3%.

The mortality rate among the lambs of this breed is rather low in spite of the fact that the estimate was biased downward. This can be taken as an indication that the dams are capable of rearing their lambs during the hazardous period until weaning. This point is of a great economic importance, especially when it is considered along with their high fertility and the rapid rates of gain of the lambs.

Heritability of Number of Lambs Born. The daughter-dam regression and the half-sib correlation methods were used in estimating the heritability of number of lambs born. In both cases the average number of lambs per lambing for each ewe was used.

A heritability estimate of .266±.108 was obtained by doubling an overall daughter-dam regression using 343 pairs.

The nature of the data did not allow for having many daughter-dam pairs within sire groups. Only 175 pairs, with an average of 2.81 per sire, were available for calculating an intra-sire regression of daughter on dam (table 7). Doubling this regression gave a heritability of .538±.143. Another estimate of .298±.330 was obtained from table 7 by multiplying the intraclass correlation (half-sib correlation) by four.

The first estimate of .266 is more reliable than the other two since it was based on a larger number of daughter-dam pairs. It should be borne in mind that this estimate was based on data not corrected for the effect of either flocks or sires. This and the inequality of the number of lambings

per ewe, must have contributed to inaccuracy of the estimate. Also estimates based on averages are expected to be higher than those based on single records. To equate such estimates to a single record basis information concerning the individual lambings and the repeatability of litter size should be available. Unfortunately, the nature of the data did not make it possible to obtain such information.

Johansson and Hansson (1943) obtained an average heritability of .214 using the daughter-dam regression based on three lambings, and .196 using the half-sib correlation based on two lambings. Desai and Winters (1951b) found that the overall heritability in several purebred and crossbred lines of sheep was .0736±.0152. In the Ossimi sheep it was .0362 as estimated by Ragab and Asker (1954). Rendel (1956) gave an average estimate of .113, while the range was from .037 to .224 depending on the breed and

TABLE 7. MEAN SQUARES AND COVARIANCES FOR NUMBER OF LAMBS BORN

Source of variation	Degrees of freedom	Mean squares		Cross-products
		Dams	Offspring	
Total	174	.14034	.11155	.03856
Between sires	61	.10213	.12672	.02984
Within sires	113	.16097	.10336	.04327

method of analysis. For mature crossbred and Navajo ewes the heritability was .22 and .12, respectively (Sidwell, 1956), and for the Rahmani ewes it was .08 (Karam, 1956).

Practical Considerations

Egypt does not produce enough meat or mutten to satisfy its needs. This shortage is met by importing large numbers of sheep and cattle from neighboring countries as well as importing frozen meat. Increasing the incidence of twinning among the indigenous breeds of sheep will undoubtedly increase lamb production and consequently lower the amount of meat imported. The heritability of twinning among the Egyptian sheep is rather low (Ragab and Asker, 1954; Karam, 1956). This would tend to make genetic improvement through selection, a slow process. Therefore, for a quick solution it was thought of introducing some of the standard breeds of sheep which proved successful in other countries. This introduction has two main objectives. First, to establish these new breeds in Egypt as good producers of mutton and wool. Second, to use their inherent characteristics of high fertility, high wool production, and rapid rate of growth in improving the indigenous breeds of sheep and probably also in constructing new breeds of better potentialities.

The Texel breed was among those recommended and introduced for this purpose. According to the Central Bureau of Sheep Breeding in 1939, the Texel sheep has several excellent qualities. The average number of lambs born per birth was 1.88 while the average number of lambs reared was 1.62. The average weight of ram lambs was 45 kg. and of ewe lambs 38 kg., at four months of age. The average weight of unwashed fleece was 5.85 and 4.57 kg. for rams and ewes, respectively. Quality of the fleece was generally 52 and 50 (Bradford system). This would be Quarter Blood by U. S. Standards.

The present study was carried out to analyse some of the above mentioned characters, namely; number of lambs born and number reared till weaning age. The two characters were found to be highly correlated phenotypically. The correlations were .68, .55, .58, .52, .57 and .67 for ewes with 1, 2, 3, 4, 5 and 6 lambings, respectively. The average number of lambs born per birth and number reared were slightly higher than those reported by the Central Bureau of Sheep Breeding in 1939. This shows that practically no progress has been made since that time. The lack of significant differences between the averages of dams and dams' dams, which was mentioned earlier, leads to a similar conclusion. Since the "heritability" of the number of lambs born was not low (.266) it appears that no selection for that character was practiced, assuming that environmental conditions did not differ much from one generation to the other.

The high number of lambs born per birth and its fairly high heritability, along with the low mortality rate of the lambs from birth up to weaning age, put the Texel sheep among the most desirable breeds for improving these characters in the Egyptian sheep. If experience proves that the Texel sheep can survive in this country without much degeneration in their merit a great revolution in Egyptian sheep production should be possible.

Summary

In 1955, 385 ewes and 10 rams of the Texel sheep from Holland were introduced into Egypt, at the Tahreer Province. This breed is known for its high frequency of multiple birth, the lambs are fast growers, and the mature animals give a heavy wool crop of medium fineness.

The records of 333 dams, 275 dams' dams, and 146 sires' dams of the imported animals showed that the average number of lambs born for ewes with 1, 2, 3, 4, 5 and 6 lambings were; 1.91, 3.66, 5.90, 7.37, 9.67 and 11.76, respectively, with an average of 1.91 lambs per lambing. The average number of lambs reared for ewes with 1, 2, 3, 4, 5 and 6 lambings were; 1.77, 3.18, 5.04, 6.44, 8.40 and 10.48, respectively, with an average of 1.67 lambs per lambing. Percent of lamb mortality, from birth until weaning age, had an average of 13.1. The "heritability" of number of lambs born was $.266 \pm .108$.

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SPERM OUTPUT AND FERTILITY OF RABBITS EJACULATED EITHER ONCE A WEEK OR ONCE A DAY FOR FORTY-THREE WEEKS

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IN the routine operation of dairy cattle artificial breeding organizations semen is collected from the bulls about once a week. Usually one or two ejaculates are obtained, which provide enough motile sperm to breed several hundred cows. Although the usefulness of outstanding sires might be further increased by more frequent ejaculation, the general belief has been that frequent ejaculation cannot be practiced for long periods of time without reducing the sperm output and fertility of bulls.

Lloyd-Jones and Hays (1918) allowed bucks to copulate 20 times in a single day and observed a decrease in semen volume, sperm concentration and litter size as the number of copulations increased. In contrast, Hammond (1921), after permitting a buck to copulate 43 times in two days and 39 times in eight hours one month later observed no decrease in litter size. Edwards (1940) ejaculated bucks 10 times a day once a week for 24 weeks and obtained an average of 351 x 10^6 sperm per week from the control bucks during the first four weeks as compared to the 437 x 10^6 sperm obtained during the last four weeks. Venge and Frolich (1951) ejaculated bucks six consecutive times at hourly intervals once a week for four weeks and observed a decrease in sperm output per ejaculate of nearly 50% during the first two weeks with a leveling off from the 2nd to the 4th week. Doggett (1956) ejaculated rabbits daily for periods as long as 36 days and observed that total sperm count rose to a definite peak every 2 to 7 days.

Dougherty and Ewalt (1941) obtained semen from one bull for 31 consecutive days without establishing any noticeable downward trend in sperm production. Weatherby *et al.* (1940) ejaculated a bull for 57 days and found no marked variation in weekly volume of semen but did observe a decrease in sperm concentration. Almquist and Hale (1956) increased weekly sperm output from 10 bulls 112% by obtaining six rather than two ejaculates per week. Boyd and VanDemark (1957) in a study of the rate of sperm production in the bull observed that sperm numbers were restored within a week after 10 consecutive ejaculates were taken within a 90 minute period.

In a short time experiment Hafez and Darwish (1956) observed slight

[1] These data were taken in part from a thesis presented by the senior author to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

decreases in sperm concentration when buffalo bulls were ejaculated at intervals of 2, 8, 12 and 24 hours.

From the literature on rabbits and bulls, and that pertaining to other domestic animals, reviewed by Anderson (1945) and by Emmens and Blackshaw (1956), it is apparent that frequent ejaculation is possible, for relatively short periods of time without serious effects on sperm output and fertility, but little is known concerning the effects of frequent ejaculation for prolonged periods of time on sperm output and fertility of males. This paper reports the results of such a study with rabbits.

Experimental Procedure

Fourteen sexually mature Dutch-type rabbits purchased from a commercial breeder were used in this experiment. Prior to the experiment all bucks had been trained to serve an artificial vagina. From this group of 14, 12 were chosen at random for use as experimental animals. Single ejaculates were collected from these 12 at weekly intervals for a 13-week preliminary period, after which they were allotted at random to two treatment groups, one group to be ejaculated once each week and the other one each day. During the experimental period it was necessary to discard from each group one buck because of lack of libido and one because of disease, thus, four bucks remained in each experimental treatment group.

The semen collections were made about 10 a.m. each day using an artificial vagina described by Macirone and Walton (1938), but modified by substituting for the plain glass cuvette a graduated centrifuge tube cut off at the 5 ml. mark. The vaginas were filled with 80° C. water and a small amount of K-Y lubricating jelly was smeared over the opening. A live doe, or a normal or castrated buck was used as a mount. The order of collection of the bucks on the once a day schedule was determined by randomization each day. Individual bucks on the once a week regimen were scheduled on different days and usually were ejaculated immediately following the collection of the bucks ejaculated daily. The bucks were weighed at each collection period.

Any gel mass present in an ejaculate was removed before determining the volume of the ejaculate. The percentage of motile spermatozoa were estimated by the procedure of Willett and Salisbury (1942) as used in this laboratory for examining bull semen. The pH of each ejaculate was determined with a Beckman pH meter, Model G, fitted with a one drop electrode. Because the "granules" in rabbit semen precludes the use of a photelometer to determine sperm concentration, a Spencer Brightline Hemocytometer was used for this purpose.

The fertility of the semen from the two groups of bucks was determined during the 37th through the 40th week of the experimental period and was based upon the conception rate and size of litters produced by does inseminated artificially after they had copulated with a vasectomized buck.

All of the does had previously borne one litter and were considered capable of normal reproduction.

It was planned to inseminate three does per buck with approximately 20 million motile sperm per insemination, but in the group sampled weekly one buck failed to ejaculate three times and second buck failed once during the 37th through the 40th week. As a consequence there were eight does bred to the bucks ejaculated at weekly intervals and 12 to the bucks ejaculated daily.

TABLE 1. AVERAGE VALUES AND STANDARD DEVIATIONS FOR SEMEN CRITERIA AND BODY WEIGHTS OF RABBITS EJACULATED EITHER ONCE A WEEK OR ONCE A DAY

Criteria	13-wk. preliminary period		43-wk. experimental period	
	Once a week group	Once a day group	Once a week group	Once a day group
Number of bucks	4	4	4	4
Maximum possible number of ejaculates based on planned frequency of ejaculation	52	52	172	1204
Number of ejaculates obtained	49	52	160	1201
Percent of ejaculates obtained containing motile sperm	100	100	88	95
Milliliters of semen per ejaculate	.45±.17	.83±.32	.43±.26	.49±.24
Percent motile sperm in ejaculates	66±14	68±7	63±8	62±8
Millions of sperm per milliliter of semen	407±252	407±209	303±169	207±145
Millions of motile sperm per ejaculate	138±110	243±181	97±88	55±38
Millions of motile sperm per week	138±110	243±181	97±88	395±266
pH	6.92±.46	6.70±.39	7.14±.42	6.90±.40
Body weight in kilograms	2.3±.35	2.4±.31	2.3±.46	2.4±.32

After completion of the 43-week experimental period the bucks were slaughtered and their testes and adrenal glands removed and weighed. Also, a cross-section 3 mm. thick of each testis and half of each adrenal gland was fixed in Bouins solution, sectioned at 8 microns, stained with hematoxylin and eosin, and examined for signs of normal and abnormal histology.

Results

A summary of the semen data for the priliminary and experimental periods for each treatment group is shown in table 1. The fact that 1201 ejaculates were obtained out of a possible 1204 from the bucks ejaculated daily suggests that males in general may be capable of frequent ejaculation

for much longer periods of time than previously has been thought possible. All but 46 of the 1201 ejaculates from the bucks ejaculated daily contained motile sperm. Thirty-five of these 46 ejaculates were from a single buck that had suffered from coccidiosis for a period of two weeks. In the group ejaculated at weekly intervals 18 of the 160 ejaculates did not contain motile sperm, 12 of which were from a single buck.

Ninety percent of the ejaculates from the bucks ejaculated at weekly intervals and 93% of those from the bucks ejaculated daily were obtained within five minutes after the teaser was placed in the bucks' cage. Furthermore, the ejaculates from the bucks ejaculated daily were obtained just as quickly during the final weeks of the experiment as at the onset.

In the semen of the bucks ejaculated at weekly intervals the average sperm concentration decreased approximately 25% and the average volume and percent motile sperm decreased approximately 5% each during the experimental period as compared to the preliminary period. The net result was that the motile sperm output per ejaculate decreased from 138×10^6 to 97×10^6 or approximately 30%.

In comparison, sperm concentration in the semen of the bucks ejaculated daily decreased approximately 50% while ejaculate volume, and percent motile sperm decreased 40% and 9%, respectively during the two periods. The net effect of these changes was to reduce the average number of motile sperm per ejaculate from 243×10^6 to 55×10^6 or about 77% for this group.

The motile sperm output per week for the bucks ejaculated at weekly intervals averaged 97×10^6 as compared to 385×10^6 for the bucks ejaculated daily. This is an increase of approximately 400% for the bucks ejaculated once a day.

In the once a day group there was a marked decrease in motile sperm output during the first six weeks of the experimental period. The week to week changes in the motile sperm output for the two groups of bucks are shown in figure 1. When the treatment means for motile sperm production per week were adjusted by covariance for the differences between groups during the preliminary period the average motile sperm output per week was 93×10^6 and 351×10^6 for the weekly- and daily-ejaculated groups, respectively, an increase of about 380% for the bucks ejaculated once a day. The difference between the two groups was significant at $P < .05$.

In both treatment groups the average pH of the semen was 0.2 units higher during the experimental period than in the preliminary period. The body weights of the bucks in both treatment groups remained essentially constant from the beginning to the end of the experiment, a period of 56 weeks.

Table 2 shows the fertility results from does bred artificially with semen from the experimental bucks after they had been ejaculated for a period of 37 to 40 weeks. The average percentage of does kindling was not significantly different as judged by the χ^2 test ($P \cong 0.7$). The difference in

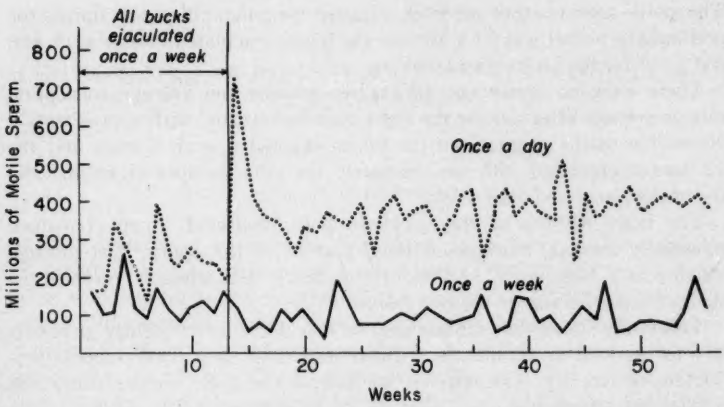

Figure 1. Motile sperm output per week of bucks ejaculated either once a day or once a week for forty three weeks.

average litter size of the two treatment groups was likewise nonsignificant ($P > .50$).

Post mortem examinations, gross and histological, of the testes and adrenals revealed no evidences of hypertrophy or degeneration of these organs.

As a consequence of these general findings with the rabbit, showing that neither sperm output nor fertility was measurably impaired by daily ejaculation for many months, the response of dairy bulls to similar treatment is being investigated.

Summary

Semen was collected with an artificial vagina from each of two groups of four Dutch-type rabbits for a period of 13 weeks. Following this 13-week preliminary period one group was ejaculated once each day and the other group continued to be ejaculated once each week for 43 additional weeks.

TABLE 2. FERTILITY OF BUCKS AFTER EJACULATION EITHER ONCE A WEEK OR ONCE A DAY FOR 37 TO 40 WEEKS

Item	Bucks ejaculated	
	Once a week	Once a day
Number of does bred	8	12
Number of does kindling	6	8
Percentage of does kindling	75	67
Total number of young born	49	65
Average litter size	8.2	8.1

The motile sperm output per week adjusted for group differences during the preliminary period was 93 x 10^6 for the bucks ejaculated once a week and 351 x 10^6 for the bucks ejaculated once a day.

There were no significant differences between the average conception rate or average litter size for the eight does inseminated with approximately 20 million motile sperm from the bucks ejaculated once a week and the 12 does inseminated with approximately the same number of sperm from the bucks ejaculated once a day.

The body weights of the bucks in both treatment groups remained essentially constant throughout the 56 weeks of the study. Post mortem weights and histological section of the testes and adrenals revealed no signs of tissue damage in the two groups.

This study shows that rabbits may be ejaculated as frequently as once a day for as long as 10 months without impairing their libido, sperm production or fertility. The response of dairy bulls under similar conditions is being investigated.

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HERITABILITY AND REPEATABILITY OF GESTATION LENGTH IN BEEF CATTLE

JOHN D. WHEAT [1] AND J. K. RIGGS

Texas Agricultural Experiment Station [2]

TWO concepts of heritability, designated as the narrow sense and the broad sense, were defined by Lush (1949). Heritability in the broad sense was defined as the fraction of the phenotypic variation caused by genetic differences of all kinds, while heritability in the narrow sense was defined to include only the fraction of the phenotypic variance caused by the average or additive effects of the genes. Repeatability is the correlation between successive records by an individual and equals heritability in the broad sense plus any environmental effects which persist over the period of observation. Repeatability can therefore, be regarded as an upper limit which heritibility in the broad sense approaches if no permanent environmental effects exist. The purpose of this study was to calculate and compare heritibility and repeatability for gestation length in beef cattle.

Data and Methods

The 608 gestation records were those analyzed by Wheat and Riggs (1952), plus additional records from the same herds. Included were 202 records for Aberdeen-Angus calves, 69 for Shorthorns, 275 for Herefords, 43 for Brahman-Hereford crossbreds, and 19 for calves sired by Brahman-Hereford sires and out of Hereford cows. The breed averages were 279.5, 281.3, 284.9, 286.6, and 286.9 days, respectively. There were 27 sires and 283 dams represented. The overall average gestation length was 282.9 with a standard deviation of 6.7 days.

Since the sex of the calf caused no significant difference in gestation length in the previous study, the data were not corrected for the sex of the calf. The males were carried almost one day longer than the females, but since each sex differed only half a day from the average of the two, the component due to sex would have been about .25. A comparison of the .25 with the component among full sibs in table 1 indicates that sex was a relatively insignificant source of variation.

It was impossible to adjust the data for year effects since the years were not recorded on all sheets where gestation length had been copied. It seems likely that if yearly differences were important they might increase the intraclass correlation for sires and decrease it for dams as these differences would probably increase the variance between sires more than that within

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[2] Paper No. TA-2536 in the Technical series.

sires, whereas the effect of years might influence the variance between dams less than that within dams.

Estimates of heritibility were calculated by multiplying the paternal half-sib correlation and the maternal half-sib correlation by four and by doubling the full-sib correlation. This treats gestation length as a characteristic of the calf. The correlations were derived from the variance components, which were obtained by equating the observed mean squares to their expectations. The intraclass correlations between gestations by the same cow when mated to different sires of the same breed and between gestations by the same cow when mated repeatedly to the same sire were used as repeatabilities of gestation length. These treat gestation length

TABLE 1. ANALYSIS OF VARIANCE OF GESTATION LENGTH FOR FIVE BREEDS OF CATTLE

Source of variation	D/F	Mean square	Expected mean square
Total	607	44.6	
Breeds	4	1138.4**	
Sires within breed	22	134.3**	E+1.37 D+20.98 S
Dams within sires	416	34.5	E+1.37 D
Full sibs	165	31.6	E

** P<.01.
E=31.6=Variance within sets of full sibs.
D=2.1=Extra variance among paternal half sibs as compared with that among full sibs.
S=4.8=Extra variance among non-sibs as compared with that among paternal sibs.

as a characteristic of the dam. Operationally, these are observed correlations between the gestation lengths of maternal sibs and full sibs, respectively.

Results and Discussion

Heritability. Significant breed differences and resemblance between relatives within a breed indicate that a fraction of the observed variation in gestation length is caused by individual differences in heredity. The analysis of variance of gestation length, with the expected mean squares, is presented in table 1.

The fact that the coefficient for the dam component in the expected mean square (table 1) for "sires within breed" is the same as the coefficient for the dam component in the mean square for "dams within sires" is coincidental as generally this is not the case for analyses involving unequal subclass numbers.

The approximate 90% fiducial limits (Bross, 1950), were 0 and 7.5 for the dam component and 2.1 and 12.0 for the sire component. The method is based on the assumption that mean squares are distributed as chi-squares, which is not the case when subclass numbers are unequal. The two components of variance should be equal unless permanent prenatal environment influences gestation length; i.e., unless gestation length were partly a

characteristic of the dam. If this factor were important the dam component would tend to be larger than the sire component. The sire component was 2.24 times as large as the dam component but, with the large overlapping of the fiducial intervals, sampling errors could have caused the observed difference in the two components. The year differences, if important, could have contributed to the larger sire component.

Heritability estimates based on the maternal half-sib correlation, the full-sib correlation, and the paternal half-sib correlation, and on the assumption of random mating within a breed and the absence of environmental correlations are shown in table 2.

Since the dam component of variance had a smaller fiducial interval than the sire component, it is possible that the .22 derived from the maternal half-sib correlation is the most reliable estimate of heritability. Though usually it is believed that the estimate based on the full-sib correlation is more reliable since any errors existing in the estimated variance components

TABLE 2. HERITABILITY ESTIMATES FOR GESTATION LENGTH

	4D	2(S+D)	4S
Method of estimation	S+D+E	S+D+E	S+D+E
Heritability estimate	.22	.36	.50

are multiplied by two rather than by four. The former estimate is considerably lower than the .38 which Jafar *et al.* (1950) reported as heritibility in the narrow sense for gestation length in Holstein-Friesians. Brakel *et al.* (1952) published significant correlations of .32 for Ayrshires and .20 for Jerseys, between the time the dam spent *in utero* and the time her individual progeny spent *in utero*.

The estimate of heritability based on the maternal half-sib correlation theoretically falls between heritability in the narrow sense and heritability in the broad sense as it includes the variance caused by the additive effects of the genes plus a fraction of the variance caused by epistatic gene action. Its interpretation depends partly on the extent to which gestation length is a characteristic of the offspring or of the dam.

Repeatability. Considering only gestation records for purebred calves, intraclass correlations for records for maternal sibs and full sibs were derived from the variance components obtained from the analyses of variance in gestations presented in tables 3 and 4, respectively. Since all cows were either Aberdeen-Angus, Shorthorns or Herefords, there were only two degrees of freedom for breeds.

The lower fiducial limit for the cow component was 4.6 and the upper limit was 12.0. The standard deviation of the intraclass correlation, .21, was approximately .04 when derived as described by Fisher (1950).

The lower fiducial limit for the cow component was 0 and the upper limit was 4.6, wheras the lower and upper limits for the sire component

TABLE 3. ANALYSIS OF VARIANCE IN GESTATION LENGTH AMONG BEEF COWS

Source of variation	D/F	Mean square	Expected mean square
Total	545	45.2	
Breeds	2	1783.9**	
Cows within breeds	258	47.8**	E+2.08 C
Within cows	285	30.6	E

** P<.01.
E=30.6=Variance caused by intracow differences.
C=8.2=Variance caused by differences among cows.
Repeatability $= \frac{8.2}{8.2+30.6} = .21$.

were 1.8 and 10.2, respectively. The standard deviation for the intraclass correlation was approximately .04.

Repeatability of gestation length should be higher for full sibs than for maternal sibs, provided the sire has any influence on gestation length. In the present analyses repeatability of records for maternal sibs and repeatability of records for full sibs were essentially the same, but repeatability of records for maternal sibs was slightly higher. There is no apparent biological explanation of why the maternal-sib correlation was higher or why the variance within maternal sib groups was less than the variance within full sib groups. However, since 72 of the full sib groups consisted of only two progeny, these results were probably caused by sampling errors.

Jafar *et al.* (1950) reported a repeatability of .29 for gestation length in Holstein-Friesians when a cow was mated to different bulls and .41 when she was mated repeatedly to the same sire. The value of .29 is not far from the present value of .21 obtained for the beef cow.

Since individual differences in gestation length are partially heritable, it appears possible to decrease or increase the length by selection. No reason is apparent for a breeder ever to practice intense selection for either long or short gestations. Benesch and Wright (1952) gave 279 to

TABLE 4. ANALYSIS OF VARIANCE IN GESTATION LENGTH AMONG BEEF COWS

Source of variation	D/F	Mean square	Expected mean square
Total	545	45.17	
Breeds	2	1783.9**	
Sires within breeds	20	139.9**	E+1.38 D+21.56 S
Dams within sires	366	36.1	E+1.40 D
Among full sibs	157	32.2	E

** P<.01.
E=32.2=Variance within sets of full sibs.
D=2.8=Extra variance among paternal half sibs as compared with that among full sibs.
S=4.8=Extra variance among non-sibs as compared with that among paternal sibs.
Repeatability $= \frac{2.8+4.8}{2.8+4.8+32.2} = .19$.

288 days as the optimum gestation length for cows and Williams (1927) wrote that the most favorable physiological duration is about 285 days. According to Williams, when the duration of gestation departs in either direction from this optimum it tends to assume a pathological character which increases as the distance from the physiological optimum increases.

Summary

Heritability and repeatability of gestation length in beef cattle were studied. The data consisted of 608 gestation records of which 202 were for Aberdeen-Angus calves, 69 for Shorthorns, 275 for Herefords, 43 for Brahman-Hereford crossbreds, and 19 for calves sired by Brahman-Hereford bulls and out of Hereford cows. The respective breed averages were 279.5, 281.3, 284.9, 286.6, and 286.9 days. There were 27 sires and 283 dams represented. The overall average was 282.9 and the standard deviation was 6.7 days.

Heritability estimates based on the maternal half-sib, full-sib, and paternal half-sib correlations were .22, .36, and .50, respectively.

Repeatability of gestation length was .21 and .19 for maternal sibs and full sibs, respectively.

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A SUMMARY OF THE MINUTES OF THE FORTY-NINTH BUSINESS MEETING OF THE AMERICAN SOCIETY OF ANIMAL PRODUCTION

H. H. STONAKER, *Secretary*

Colorado State University, Fort Collins

The meeting was called to order in the George Bernard Shaw Room of the Hotel Sherman, Chicago, Illinois, on November 30, 1957, at 3 p.m. by President J. C. Miller. The minutes of the 1956 Business Meeting were not read but were approved as published in the Journal of Animal Science, Vol. 16, No. 1, pp. 249–260.

The report of the Secretary, H. H. Stonaker, was as follows:

"Membership in the American Society of Animal Production increased to 1,697 in 1957. There were 103 new members with a net increase of 86 in total membership. The membership is international in nature with the following distributions: U.S.A. and Canada, 1646; other countries, 51. Through financial assistance and encouragement from the International Cooperation Administration, reduced rates are being offered a number of animal scientists in foreign countries. This should serve to increase foreign membership. The Membership Committee and the Membership at large are to be congratulated and encouraged for their important contribution to the Society.

"Subscriptions to the Journal of Animal Science increased 172 to a total of 1,382. Six hundred forty-one journals go to the United States and Canada, 766 go to 72 foreign countries. Twenty-two additional countries were represented this year among subscribers.

"There were 190 papers accepted for presentation and 62 papers read by title for the 1957 program. The Nutrition and Physiology sections continue to have many more excellent papers submitted than can be presented in the limited time.

"The new Secertary wishes to express his appreciation to Dr. Beeson for the orderly and thorough manner in which the business of the Society was transferred".

President J. C. Miller reported the registration for the 1957 meeting as being, 1,160, as compared with 1,050 in 1956 and 1,000 in 1955.

The Treasurer of the Society, H. H. Stonaker, presented the following report:

"As of October 31, 1957, the Society had total assets of $45,705.21. Of this total, $16,554.07 were in demand deposit, $10,532.24 in savings deposit and $18,618.90 were in United States Bonds.

"The balance in the Morrison Award Fund was $4,235.62, leaving $41,469.59 in the General Society Fund.

"Expenditures for 1957 were $28,311.74 and income was $30,515.41, with an excess of income over expenses of $2,201.67. This compared to $5,651.59 increase in 1956. The difference was due largely to a lowered return from reprints and increased journal costs".

President J. C. Miller read part of the Auditing Report and stated that the detailed records are on file in the Treasurer's Office for examination. The audit for the year ended October 31, 1957 was made by the firm of Rogers and McCluskey, Certified Public Accountants, Fort Collins, Colorado. President Miller read the following from the statement of Robert R. McCluskey, Certified Public Accountant.

"We have examined the Balance Sheet of the American Society of Animal Production as of October 31, 1957, and the related statements of income and expense for the fiscal year then ended. Our examination was made according to generally accepted auditing standards and accordingly included such tests of the accounting records and other auditing procedures as we considered necessary in the circumstances.

"In our opinion, the accompanying Balance Sheet and related statements presents fairly the recorded financial position of the American Society of Animal Production as of October 31, 1957, and the results of its operations for the year then ended".

The Business Manager's Report was as follows:

"A total of 3,300 copies of the Journal were printed quarterly with an average total cost of $1.49 per copy. Subscriptions, memberships and reprint sales provided approximately equal sources of revenue for the printing of the Journal.

"The records of the Business Manager listed the following back numbers of the Journal and Proceedings available for sale on November 1, 1957.

JOURNAL OF ANIMAL SCIENCE

Vol.	Year	Complete volumes	Number of copies in single issues			
			No. 1	No. 2	No. 3	No. 4
2	1943	0	11	0	0	0
9	1950	0	0	0	37	41
10	1951	0	0	82	0	30
11	1952	0	0	0	65	121
12	1953	28	48	82	67	28
13	1954	264	264	285	301	367
14	1955	40	67	83	91	40
15	1956	82	82	121	149	460
16	1957	228	232	228	244	245

PROCEEDINGS OF THE SOCIETY

Year	No. of copies	Year	No. of copies
1915–16	46	1931	55
1922	324	1932	17
1923	239	1933	35
1925–26	43	1934	23
1927	45	1935	25
1929	28	1940	40
1930	26		

"These back numbers may be purchased from the Business Manager at the following rates: Volumes 10–15—$2.00 per copy; preceding issues—$1.25 per copy; proceedings—$1.00 each. Add 25 cents for all foreign deliveries".

The Editor of the Journal of Animal Science, J. K. Loosli, gave the following report:

"In Volume 16 of the Journal of Animal Science, the Society published 126 scientific papers, four review papers presented as a symposium on the meat type steer, and 246 abstracts of papers read at the annual meeting. The 1,130 pages of Volume 16 also included proceedings of the 1956 Annual Meeting, a News and Notes section in each issue, and author and subject indexes for the original articles published and for the abstracts.

"A total of 170 manuscripts have been received since November 15, 1956. Of these, 100 have been published or are now at the printers for the February 1958 issue, 13 have been rejected, 24 are being revised by the authors, and 33 are in the hands of the reviewers.

"The terms of A. V. Nalbandov and C. E. Terrill as members of the Editorial Board expire on December 31, 1957. Sincere thanks and appreciation are extended for the many hours of service they have given in reviewing manuscripts and advising authors in the interest of improving the quality of the Journal. The Executive Committee of the Society has appointed R. O. Berry, Doyle Chambers and T. W. Perry to the Editorial Board, effective January 1, 1958. Appreciation and thanks are also extended to the other members of the Editorial Board, to W. D. Gallup, the Associate Editor, and others who have assisted with the editorial work.

"I personally wish to thank members of the Society for their cooperation and tolerance and for their willingness to overlook the mistakes I have made. I hope everyone will extend this same spirit of cooperation toward W. D. Gallup, the new Editor of the Journal".

The report of the Nominating Committee was given by J. I. Miller, Chairman: W. M. Beeson was nominated for President; H. H. Stonaker was nominated for Secretary-Treasurer; Rufus Cox and W. P. Garrigus were nominated for Vice-President.

F. B. Morrison moved that the Secretary be instructed to cast a unanimous ballot for the President and Secretary-Treasurer. Craft seconded. Carried. Ballots were distributed for voting by Tellers K. F. Warner, G. W. Salisbury and T. A. Dowe.

Report of the Committee on Research was given by E. J. Warwick:

"The Research Committee has reviewed four sections of the proposed monograph and has made specific comments and suggestions to the Monograph Committee. After consideration of modifications to meet these suggestions and consideration by the Monograph Committee of the possibility of developing additional sections on other phases of nutrition, we recommend publication.

"The question of standards for beef carcass grading continues to be a live one, and during the year the Executive Committee requested that the Research Committee consider reviewing the available research evidence and consider recommending changes if the evidence warranted this. Most people recognize the limitations of present beef carcass grade specifications but we feel research evidence is not now available to permit an adequate response to the request of the Executive Committee. Rather, we recommend that renewed emphasis be placed on this phase of research, especially the relationship of conformation, quality and finish, individually or collectively, to eating quality of the meat. When data become available, proving changes are needed, we recommend that this Society review the evidence and make recommndations".

The report of the Monograph Committee was given by C. E. Terrill:

"The monograph, 'Techniques and Procedures in Animal Production Research' is now complete except for some revisions. It is planned to complete these revisions and to submit the manuscripts for publication as soon as possible".

L. E. Washburn stated that the Teaching Committee had no special report to make to the Society, but it was recommended that invitational papers be used to make up the 1958 program.

No formal report was made by the Extension Committee. Report of the Membership Committee was given by W. M. Beeson:

"There have been 169 new members join the Society this year. The distribution of new members on a regional basis is as follows: Western Region, 41%; Midwest Region, 21%; North Atlantic Region, 20%; Southern Region, 12%; and Foreign, 6%.

"I should like to express my sincere appreciation to the members of this committee for the execllent cooperation and job that they have done in the past year in obtaining new members for the Society".

The following report of the Committee on Information was given by Carl F. Neumann:

"This report will cover two phases of the work of the Committee on Information —the highlights of last year's meeting, and a preliminary report of this year's activities.

"First—Last Year. We do not presume to know about all of the publications which may have used news material concerning the 1956 meeting, since we do not have access to all of them.

"We do know, however, from checking that has been done, that news relative to the program of last year's meeting filtered out into states across the nation. News stories were used in daily and weekly or livestock, farm and market papers from coast to coast, including the states of Illinois, Iowa, Indiana, Michigan, Ohio, Massachusetts, Virginia, Missouri, Minnesota, Nebraska, North Dakota, South Dakota, Texas, Montana, Arizona, California, Colorado, Louisiana, Oklahoma, New Mexico, Kansas, Georgia and New York.

"Stories appeared in such daily papers as the Chicago Daily News, Chicago Tribune, Cedar Rapids Gazette, Evansville, Indiana, Courier; and the Anderson, Indiana, Herald. They came out in such farm publications as the Farmer of St. Paul, Minnesota; the Kansas Farmer, the Ohio Farmer, Michigan Farmer, Nebraska Farmer, and the Prairie Farmer of Illinois.

"News material emanating from last year's meeting was noted in such livestock papers as the Hereford Journal, Shorthorn World, Aberdeen Angus Journal, Breeder Stockman, the Cattleman, Montana Wool Grower, New Mexico Stockman, National Live Stock Producer, Bershire News, Yorkshire Journal, Southern Livestock Journal, Hampshire Herdsman, Breeders Gazette, and others. Among the market papers using material were the Chicago Drovers Journal, Omaha Journal Stockman, Kansas City Drovers Telegram, St. Louis Livestock Reporter, Western Livestock Journal, St. Joseph Livestock Journal.

"So much for last year. The work this year is underway. News stories are being mailed out to 32 cattle publications, 10 market papers, eight swine publications, six sheep journals and 19 farm papers, two meat magazines, and a few miscellaneous publications.

"In addition, news stories have been furnished farm radio directors of Chicago radio stations, to Chicago daily papers and to the press wire services. The latter

includes the Associated Press, United Press and International News Service, and they relay selected news to papers throughout the country.

"We can of course never predict the possible use of material. We do know, from previous experience, that editors will not use stories that they feel are too scientific, regardless of their possible merit. Editors do not want stories which are overly long. They like stories which tell of work done which they believe has a practical application on farms and ranches. These facts are in no way designed to cast any reflection on the scores of fine papers which have been presented at this meeting—all of which, we are sure, serve a very useful purpose.

"If this Committee, whose members also include Howard Biggar, J. K. Loosli, and J. O. Grandstaff, accomplishes even in small measure its goal of letting more and more people know about this Society, its program, and something about the findings of its members in the research or other fields, it feels that its efforts may be of some value. Thank you".

George W. Litton reported for the Necrology Committee and recommended that the following memorials be published in the Journal of Animal Science: Edwin Stanton Good, Henry William Vaughan, D. P. Waldorf, and Alfred Leroy Baker.

This report was accepted with a minute of silent prayer in remembrance of these members.

Reports of Special Representatives of the Society

T. C. Byerly read an invitation from the American Association for the Advancement of Science to attend the symposium for Section O—Agriculture, on the Control of Plant and Animal Pests by Chemicals and Genes.

J. E. Foster reported on the activities of the Committee on Intersociety Cooperation and Scientific Manpower Commission:

"Since the third annual report given at the business meeting of the Society last year, meetings have been held on February 6, May 16, October 31–November 2 and November 22. The one in May was a joint meeting of the Engineering and Scientific Manpower Commissions and was held in New York. The October 31–November 2 meeting was a conference on Engineering and Scientific Education held in Chicago, at which Dr. J. B. Outhouse of Purdue University represented our Association. At the Scientific Manpower Commission Meeting on November 22, your representative was nominated for Secretary-Treasurer. Reports of these meetings have been made to your Secretary.

"At the February meeting a report was made on the current military manpower situation, which was briefly as follows:

"(1) The administration of Selective Service regulations is eminently satisfactory at the national level, but decisions at the state and local levels still require a great deal of monitoring. Many local boards are again calling graduate students for induction particularly those who have reached ages 24 and 25.

"(2) There is a surplus of ROTC trainees in the Army, and an effort is being made to fill the quotas for two-year servicemen by calling for volunteers from the entire graduate group. The number of volunteers is not quite sufficient to fill the quota, particularly in the Corps of Engineers, and the vacancies are

being filled by lot. EMC and SMC believe that engineers and scientists should be given preference for routing into the six months' reserve program and that vacancies in the two-year service program should be filled from the ranks of nonscientific and nonengineering ROTC graduates.

"(3) Assistant Secretary of the Army Hugh Milton is making an earnest effort to improve the administration of the Scientific and Professional Program in installations where administration has been notoriously poor. There has not been time for a dispassionate judgment on the results of his efforts.

"(4) Since the administration of the Army regulation on early release (AR 635–205) has been placed in the hands of the Adjutant General (May 13, 1956), the regulation has become meaningless and completely ineffectual. The Commission heartily endorsed the efforts of its Executive Director to have the situation corrected, however drastic necessary action may become.

"The Executive Director transmitted a suggestion from Alan Gamble, who is in charge of the Register of Scientific Personnel at the National Science Foundation, that SMC explore ways and means of obtaining a higher ratio of registrations of scientists with those of its nominating agencies that are on contract with the National Science Foundation. In the discussion that followed it was learned that several of the nominating agencies communicate the details of their methods and experience for transmittal to Dr. Gamble.

"The commission has taken grave consideration of our present and future status of properly trained scientific personnel. Among the items that came up for consideration were: specialization in college, early training, need for expanded college facilities, support of education, and scholarships. The supply of trained personnel is greater now than in the past but the selection quality-wise is much more critical.

"Although Congressional and public interest has been stimulated by a couple of 'Sputniks', it is by no means certain that corporate interest has similarly been aroused to the point where 1958 contributions will support the 1958 program of SMC. It was therefore recommended that SMC's nominating agencies be asked to renew their contributions to the Commission for 1958.

"*Accomplishments*

"(1) Reversal of the marked downward trend in engineering and scientific enrollments in the early 1950's. This was an effort to prevent future shortages of graduates by encouraging qualified high school graduates to consider the opportunities in engineering and science.

"(2) Improvement of military manpower policies involving engineers and scientists.

"(3) EMC and SMC have been successful in their efforts to maintain and even expand the programs for occupational and student deferments. There is little doubt that without these efforts these programs would have been seriously crippled.

"(4) EMC and SMC were successful also in the development and improvement of the Army Scientific and Professional Personnel Program. In this area, the Commissions provided the outside impetus necessary to improve procedures to identify and classify qualified engineers and scientists. Although the problem of the utilization of these people in the professional program has not yielded as readily, there is no doubt that our efforts in this area have, on the whole, been constructive and have led, in general, to improved attitudes toward the utilization of these persons in an enlisted capacity.

"(5) Contributed also to the development of sufficiently strong counter opinion to stall and eventually stop—for the time being at any rate—the trend in the ROTC program to branch general training and the discontinuance of technical curricula.

"(6) The fundamental revision of plans for and handling of the Military Reserve Forces in time of mobilization. The automatic screening system (in practice about a year) represents a tremendous improvement in this area and the accomplishment of a specific objective of the Commissions stated in 1952.

"(7) The development and implementation of the Six Month Critical Skills Program. Operating in phase with the Occupational Deferment Program, this Critical Skills Program provides an interim answer to many vexing problems and is, possibly, the first step in the development of military service plans for engineers and scientists as such.

"(8) The development of advisory committees to the Selective Service local boards.

"(9) The development of a new flexibility in the assignment of ROTC graduates.

"(10) Growing participation of EMC and SMC in manpower plans for future mobilization. This is accomplished through a membership on the Committee on Specialized Personnel and of participation in the Executive Reserve of the Office of Defense Mobilization.

"(11) Provided the basic rationale and broad guidelines for improved utilization of engineering and scientific manpower in industry.

"(12) Improved public awareness of the importance of scientists to our national well-being.

"(13) The development of higher public awareness of the relationship between elementary and secondary education and the development of professional careers in science.

"(14) Provided a clearinghouse for information on scientific manpower problems.

"(15) Through membership on various government committees and through the consulting services of the staff of EMC and SMC the viewpoint of the scientific societies has been brought to bear on many issues. This has involved extensive work with the Bureau of Labor Statistics of the Department of Labor, the National Science Foundation, the Department of Defense, the Selective Service System and other interested agencies to insure the development of a realistic policy and its proper administration".

J. O. Grandstaff reported on the activities of the Division of Biology and Agriculture, National Research Council and Agricultural Research Institute, as follows:

"The Agricultural Board now has 16 standing committees, altogether drawing upon the experiences of 225 scientists.

"The need for additional funds to finance the activities of the various committees continues to be a rather important problem. The Board has established a Ways and Means Committee which will undertake to devise methods of bringing the work of the Board to the attention of potential donors, seek out sources of support, and help to develop procedures for getting needed funds. The Board has continued to revise and reissue its Reports on the Nutrient Requirements of Domestic Animals. The following summarizes the current status of reports developed by the Committee on Animal Nutrition:

"1. Nutrient Requirements of Dairy Cattle, revised in 1956 and issued as publication 464.

"2. Nutrient Requirements of Sheep, revised in 1957 and issued as publication 504.

"Copies may be obtained from the National Research Council at a cost of $1.00 each.

"The Reports on Nutritional Requirements of Beef Cattle, Swine, Horses, Poultry, and Laboratory Animals are being revised for publication in 1958.

"Other committees of the Board which expect to have reports ready for publication during the coming year include the following:

"1. Committee on Hormones in Livestock Feeding.

"2. Committee on Feed Additives.

"3. Committee on Animal Breeding.

"4. Committee on Range and Pasture Problems.

"5. Committee on Feed Composition.

"The latter report will include extensive data on the composition of domestic cereal grains and forages.

"During the past year a total of 63 applications for Fulbright Fellowships in Agriculture were screened and 18 full grants were made. The grants included five in the animal sciences and 13 in the plant sciences.

"The first edition of the Handbook of Biological Data compiled by a special committee of the Division of Biology and Agriculture under the chairmanship of Dr. T. C. Byerly, was published in November 1956 by the W. B. Saunders Company. The book is now in its second printing. The Committee on the Handbook of Biological Data was authorized to continue until June 30, 1961, to supervise production of a second edition. A special effort will be made to find out what is missing in the first edition that should be included in the second. In order to provide for better treatment of "growth and reproduction" in the second edition, an effort will be made to obtain additional funds for collection of data from which suitable tables for the handbook can be extracted.

"The Institute of Laboratory Animal Resources is primarily concerned with the improvement of animal stocks used in laboratory research and teaching. Standards for the production, accreditation, and certification of laboratory animals are being developed in cooperation with breeders. The Institute also serves as a central clearing house for information on laboratory animals. A greatly enlarged second edition of the Handbook of Laboratory Animals is scheduled for publication in the near future. The book is divided into 10 sections: Genetic Standards, Caging, Nutrition, Infectious and Parasitic Diseases, Primates, Housing, Supply, Germ-Free Animals, Education of Animal Technicians, and the Procurement of Animals from Nature.

"The major activities of the Food and Nutrition Board, outside of the Food Protection Committee, have been centered on problems of protein and fat nutrition. The Board is administering a grant of $250,000 from the Rockefeller Foundation to promote and support research in the production and testing of sources of high quality protein in areas of the world where protein malnutrition is important. Supported by a grant of funds from the Nutrition Foundation, the Committee on Fats is developing reports on the role of fats in nutrition. The Food Protection Committee has completed two major projects with publication of reports on the safety of surfactants for use in foods and use of pesticides in food production. Recently two new *ad hoc* committees have been established. One will serve as an advisory committee on residues and tolerances of methoxychlor in milk, and the other will serve in an advisory capacity to a subcommittee of the Congress on chemical additives in foods.

"The 6th annual meeting of the Agricultural Research Institute was held October 14–15, 1957, at the National Academy of Sciences. As a supporting agency of the Agricultural Board, the ARI continues to provide valuable counsel on agricultural problems needing attention, as well as furnishing the principal basic financial support of the Board, which is derived from annual dues of industry members. Class A membership numbers 75, a gain of 7 new industry members during the past year. Class B membership now numbers 97, including 25 scientific societies, 21 Federal agencies, 44 State agricultural experiment stations, and 7 other organizations.

"The major features of this year's program were panel discussions on utilization research, basic research, and weather and climate in relation to agriculture. The part

dealing with basic research included (1) discussions of fundamental research from the viewpoint of industrial management and a public research agency, and (2) a panel on basic research in animal sciences. Papers concerned with basic research on swine, ruminants, and poultry were presented respectively by Damon Catron, Iowa State College; J. T. Reid, Cornell University; and E. P. Singsen, University of Connecticut.

"In other sessions consideration was given to problems of agricultural public relations and future manpower.

"C. L. Rumberger of the H. J. Heinz Company was elected President of the Institute for the coming year".

In the absence of Ralph W. Phillips, J. C. Miller read the report from Dr. Phillips on the European Association of Animal Production:

"The European Association for Animal Production is now in its eighth year of activity. It has 21 member organizations from countries in Europe and the Mediterranean area. During the year under report there was no addition to the membership. Among the European countries, only Greece, Ireland, and Norway are not represented by associations in the overall European Association.

"The Association had originally six Study Commissions. This year the Commission on Research Co-ordination has been dissolved and a new Commission, the Study Commission on Genetics, has been created. The six Study Commissions had their meetings in Luxembourg at the end of June, 1957.

"The Study Commission on Sheep and Goat Production continues to give attention to subjects such as cutting of sheep carcasses, meat production and distribution, milk and butterfat recording, and an enquiry into goat production in the Mediterranean countries. A Working Group of this Commission held a meeting in Israel earlier in the year, where the problems of sheep production as effected by intensification of agriculture and fragmentation of land holdings in the Mediterranean area were discussed.

"The Study Commission on Horse Production has conducted a limited study which shows that, although mechanization of agriculture had caused a downward trend in horse stocks, there is no cause for breeders to be alarmed at this tendency as they should be able to adapt themselves to the changed circumstances. There is likely to be a change in the quality of the horse aimed at better adaptation to present day requirements.

"The Study Commission on Beef Production continued to review the work on the organization of progeny test for beef cattle. Another important topic under review is the planning of cattle improvement under artificial insemination schemes.

"The Study Commission on Animal Genetics was inaugurated under the chairmanship of Prof. I. Johansson of Sweden. Two important papers were discussed at its meeting, one by Dr. H. Robertson of Edinburgh, and one by Prof. Sorensen of Copenhagen. Dr. Robertson, reporting from his studies in England, opined that at present artificial insemination is merely an appendage to pedigree breeding and is contributing little to improve the standard of bulls entering artificial insemination schemes. Prof Sorensen reported on the need of cooperation between groups of workers in different countries in the study of blood groups of farm animals. The delegates agreed to proposals to secure closer cooperation among laboratories in Europe engaged in blood grouping work. The aims are to standardize techniques, to keep reagents for blood grouping more uniform, and to introduce a universal nomenclature.

"The Study Commission on Pig Production has worked for several years on the question of standardization of pig progeny testing methods in Europe and held a preliminary meeting in Paris in November 1956. On this occasion delegates from 14 countries measured 200 pig carcasses in a slaughterhouse. On the basis of the preliminary work, the Food and Agriculture Organization of the United Nations (FAO)

and EAAP held a joint meeting in Copenhagen at the beginning of July 1957 with the participation of representatives from 15 European countries. The conclusions of this meeting have been presented to the interested Governments. Contributions to the discussion by Dr. J. L. Lush, of the American Society of Animal Production, who was present as an observer, were highly appreciated.

"The work on the Glossary of Animal Husbandry Terms (in English, French, German and Spanish) which is a joint work of FAO and EAAP, is now in its conclusive phase. The manuscript is being prepared and it is hoped it will be ready for final editing by the end of this year. In that case, the glossary may be printed in the spring of 1958.

"In addition to reporting on the activities of EAAP, Dr. Phillips called attention to the fact that, since he is no longer stationed in Rome, he could not continue to maintain contact with EAAP on behalf of the American Society of Animal Production. The Society would, therefore, need to consider if another of its members who is stationed in Europe should be asked to undertake this assignment".

J. L. Lush moved that we continue our representation in the European Association for Animal Production. W. A. Craft seconded. Carried.

J. L. Lush reported that the European Association for Animal Production would meet during the dates of June 23–27, 1958, in Brussels. The topic is to be Productivity in Farm Animals. American members are urged to attend.

"Forty-seven (47) applications were submitted for the fellowships in all categories. Mr. Earl E. Lister of Cornell University was chosen as the Animal Husbandry recipient. He advised in April that he had just been offered a Canadian National Research Council scholarship which he accepted. Subsequently, Mr. Jack E. McCroskey of Oklahoma State University, who was first alternate, was notified that he would receive the Animal Husbandry award for the 1957–1958 school year.

"The committee recommended that the stipend be increased from $1620 to $1800. This has been approved by the Ralston Purina officials."

The following actions were taken:

J. C. Miller appointed a sub-committee for study of time and place of meetings, in response to a resolution from the Western Section of the American Society of Animal Production requesting that a vote of the membership on this topic be taken in 1958. The Committee, composed of J. K. Loosli, Chairman, Charles Hobbs, J. F. Kidwell, G. Vander Noot and A. L. Neumann, is to prepare a questionnaire by June so that the Secretary-Treasurer can ballot the membership at that time. A report on this study is to be made at the 1958 Business Meeting.

J. C. Miller presented the information that 1958 marks the 50th meeting of the American Society of Animal Production and indicated that the Executive Committee would be pleased to take into consideration recommendations concerning the commemoration meeting.

In discussing the question of meeting time, H. H. Cole moved that it is the sense of this meeting that the Executive Committee of the Society proceed to take whatever steps are necessary to increase the time allotment for the Annual Meeting to a minimum of two and a half days at the earliest

possible time, if possible, for the 1959 meeting. L. E. Harris seconded. Carried.

W. J. Loeffel reported for the special committee for the selection of Fellows of the American Society of Animal Production:

"1. The Committee recommends that the Society establish the title of Fellow for persons who have rendered very distinguished service to the field of Animal Industry over a period of at least 25 years.

"2. That they consider selecting one person per year from each of the following five categories: teaching, extension, research, administration and industry, and not to exceed three additional, irrespective of category.

"3. That persons designated as Fellows be presented an appropriate certificate or scroll.

"4. That the Executive Committee arrange for the selection of Fellows to be honored in a manner which they deem appropriate.

"5. Any member of the Society shall be afforded the opportunity of making a nomination or nominations in writing to the Secretary. The committee may be guided by but not limited to the nominations so made. Approved by committee members present".

W. J. Loeffel moved adoption of report. Howard Bogart seconded. Motion carried.

T. W. Dowe, Chairman of Tellers, reported that W. P. Garrigus was elected Vice President.

G. A. Branaman gave the following report of the Pasture and Forage Committee:

"One of the assignments of this committee during 1957 was to assist in arranging a Joint Symposium with the Agronomy Society, Dairy Science Association and Society of Range Management. The time and place selected is August 1958, at Madison, Wisconsin, during the American Society of Agronomy annual meeting.

"Chairman of Forage Committees in the Societies are: Dr. P. G. Woolfolk, Animal Production; Dr. J. T. Reid, Dairy Science and Dr. O. E. Sell, Agronomy. The Society of Range Management has not expressed definite intention to participate.

"It has been agreed that invitational papers will be arranged on topics to be selected by the Joint Committee, and which will fit into a one day program, probably on Monday of the week of the Agronomy meetings.

"A proposal has been made that the Manual of Pasture and Range Evaluation Techniques be revised by Joint Committees of the different groups. If such a procedure is undertaken, it will probably require about two years time of a continuing committee.

"It is recommended that the Pasture and Forage Committee follow up on this proposal and report to the Executive Committee".

J. C. Hillier gave the following report for the Resolutions Committee:

"BE IT RESOLVED, that the Society interest itself in Animal Husbandry Curricula in the light of employment requirements and opportunities for Animal Husbandry graduates and that a report be prepared annually for publication with the proceedings of the Society. It is suggested that this be accomplished through the Standing Committee on Teaching.

"BE IT RESOLVED, that the Society go on record as commending Dr. J. K. Loosli for three years of faithful and competent service to the Society as Editor of the Journal of Animal Science.

"BE IT RESOLVED, that the Society express appreciation to Prof. and Mrs. F. B. Morrison for their continued interest and generosity in providing the Annual Morrison Award.

"BE IT RESOLVED, that the Society express appreciation to the American Feed Manufacturers Association for its continued interest in and recognition of outstanding research accomplishments of Society members.

"In order that potentially useful germ plasm from other lands may be introduced, and tested for value in the United States, we urge that attention be given to surmounting the health problems involved and that efforts be made to activate a planned program of animal or semen introduction and testing as soon as research can develop adequate animal health safeguards. BE IT RESOLVED that the U.S.D.A. be requested to emphasize this kind of a project.

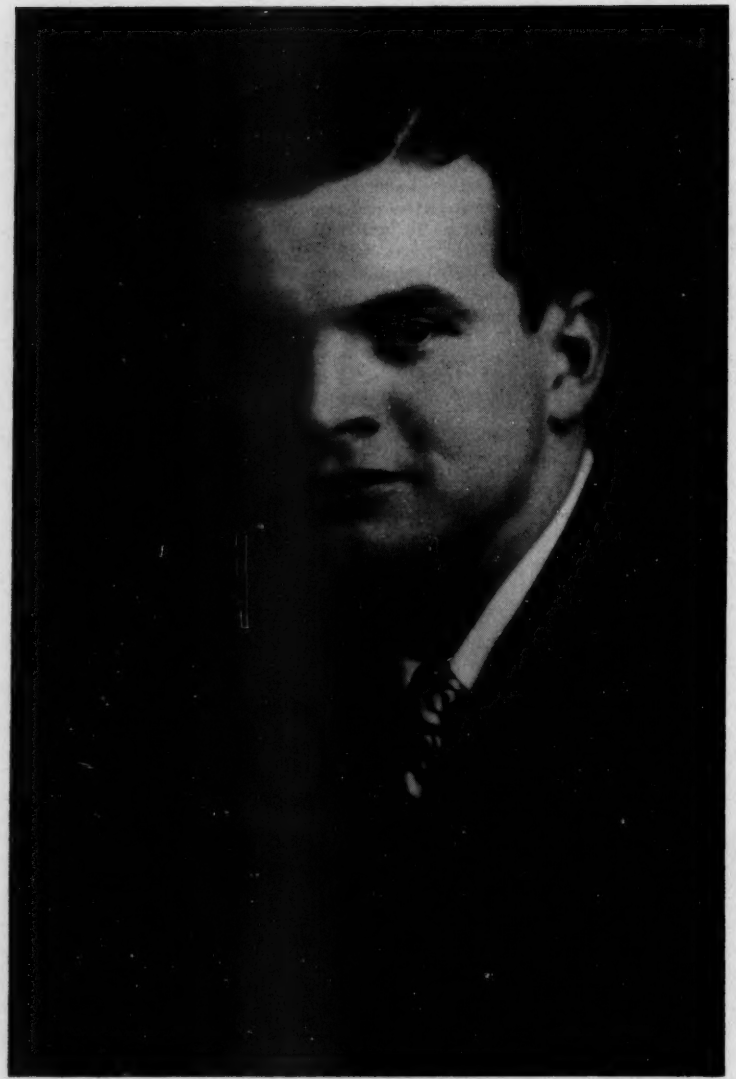
"BE IT RESOLVED that the Society express its appreciation to Mr. Carl Neumann and Mr. H. H. Biggar of the National Livestock and Meat Board for their time and effort in publicizing so well the activities of the Society.

"BE IT RESOLVED that the Society express appreciation to the Hotel Sherman for the increased meeting facilities provided".

The meeting adjourned at 4:50 p.m.

Respectfully submitted

H. H. STONAKER, *Secretary-Treasurer*



DR. D. E. BECKER

D. E. Becker was presented the tenth award of the American Feed Manufacturers Association at the 49th Annual Meeting of the American Society of Animal Production on November 30, 1957.

AMERICAN FEED MANUFACTURERS ASSOCIATION AWARD

1957

The winner of the American Feed Manufacturers Award in livestock nutrition for 1957 is another winner characterized by persistent, thorough and thoughtful research in nutritional problems of livestock production.

Donald Eugene Becker was born at Delavan, Illinois on February 2, 1923. He attended his state university, where he was recognized as an outstanding student, graduating with high honors in 1945. In 1947 he received the Master of Science degree in Animal Husbandry from the University of Illinois and in 1949 the Doctor of Philosophy degree in Animal Nutrition from Cornell University.

He is a member of Alpha Zeta, Gamma Sigma Delta, Phi Sigma and Sigma Xi.

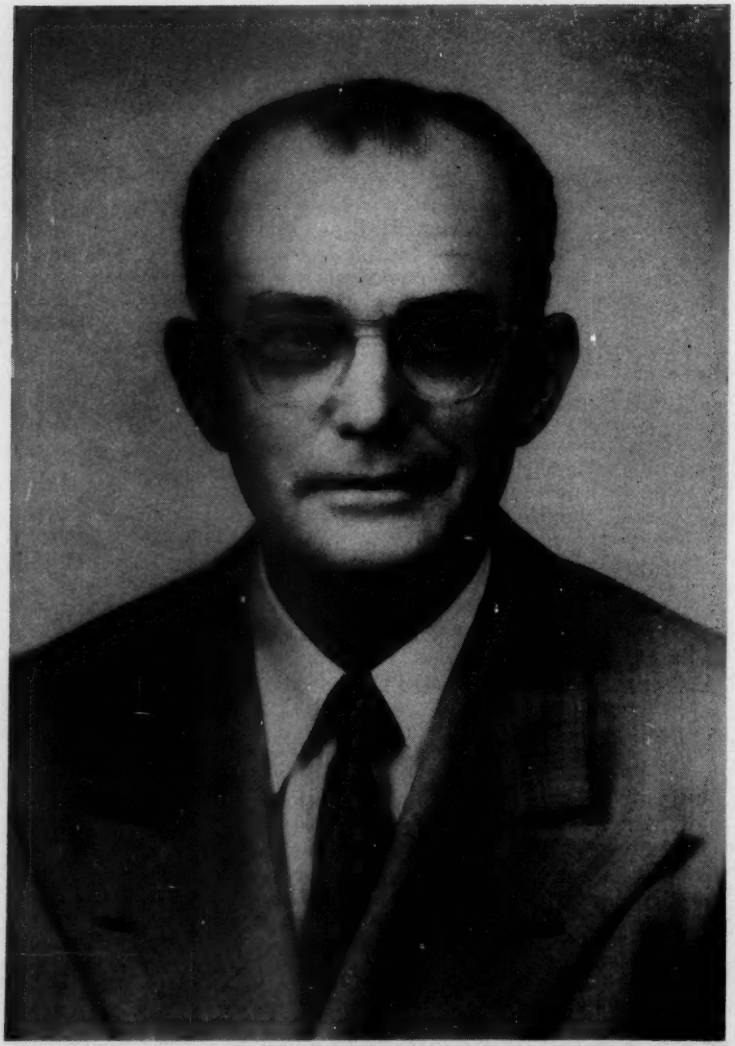
Dr. Becker has had experience in teaching and research as a part-time assistant at Illinois in 1945–47, at Cornell 1947–49, and as Associate Professor at the University of Tennessee 1949–50. From 1950 he has served as Assistant Professor and as Associate Professor at the University of Illinois where he is currently located.

This year's winner is author or co-author of 46 scientific papers in sheep and swine nutrition. He has made extensive studies of the protein and amino acid requirements of the growing pig and of nutrient interrelationships. He demonstrated the limited capacity of pigs under one week of age to utilize dietary sucrose.

His research has included the effect of limited feeding and of feeding fibrous feeds on carcass quality of swine. He has also studied the value of whole whey powder and of alfalfa meal as sources of unidentified growth factors in swine rations. He has actively investigated the place of antibiotics and of arsenicals as additives to swine rations.

Dr. Becker is highly regarded and recognized for his knowledge and critical analysis of scientific literature in his chosen field. His associates frequently consult him regarding research problems. This continuing command of available information and his creative thinking combine to make him a remarkable and productive researcher.

It is a real professional and personal pleasure for me to present to you the winner of The American Feed Manufacturers Award in livestock nutrition for 1957, my colleague and fellow Illini, Dr. Donald Eugene Becker.



DR. PAUL H. PHILLIPS

Paul H. Phillips received the twelfth Morrison Award which was presented at the 49th Annual Meeting of the American Society of Animal Production on November 30, 1957.

THE MORRISON AWARD

1957

"A rare combination of nutrition expert, chemist, histologist and farmer", was the description once given by a noted fellow scientist of Paul Horrell Phillips, recipient of the 1957 Morrison Award.

Because of his diversified interests and versatility as a researcher, he has been active in an unusually broad field of nutrition investigations. He and his associates have published well over 200 scientific papers, and he has directed the graduate training of over 50 Ph.D. candidates.

Dr. Phillips was born in Johnston City, Illinois, in 1898. He received his undergraduate training at Washington State College.

From 1926 to 1929, he accepted an assignment with the Near East Relief program in the Caucasus, where he managed extensive farming operations and trained boys in vocational agriculture. Returning to his homeland he continued his formal training at the University of Wisconsin, where he received his Ph.D. in Biochemistry and Animal Husbandry. Since completion of his doctorate in 1933, he has served continuously as a researcher and teacher on the Biochemistry staff of the University of Wisconsin.

Among Dr. Phillips' many contributions, the most noteworthy is perhaps his studies on the preservation and metabolism of sperm, which led to the development of the egg yolk buffer, and opened the door for field application of artificial insemination of cattle.

His studies on mineral metabolism have been extensive. A major contribution in this area has been his investigations on fluorine toxicity in dairy cattle, the results of which have had special application in solving contamination of grazing lands, caused by air pollution from surrounding industrial plants. Other mineral research includes the role of sodium chloride, potassium, manganese, phosphorus, cobalt and zinc in farm animal nutrition.

He has given special emphasis to the nutrition of young, which includes chemical analysis of colostrum and milk cattle and swine; the utilization of the globulin fraction of milk by the young calf; the carotene and vitamin A requirements of calves and pigs; and the formulation of "milk substitutes" for young weaned at an early age.

Dr. Phillips' research has contributed to knowledge of the relation of nutrition to several infectious and noninfectious diseases; namely, parakeratosis in swine, hyperkeratosis or X-disease in cattle, brucellosis in cattle, dental caries and calcinosis in the cotton rat. He also investigated the relationship of nutrition to parasitism in sheep. Other research includes vitamin B_{12} for sheep and swine, antibiotics as growth stimulants and cariogenic factors in grains.

Dr. Phillips is also an outstanding farmer and breeder of Holstein cattle, and he has been active in numerous community and area livestock improvement programs. Thereby, he has made available his wealth of knowledge to Wisconsin farmers in a personal and direct manner. The combination of his fundamental knowledge of nutrition and his familiarity with livestock production has made him a most popular and effective teacher.

MEMORIALS

Edwin Stanton Good

Emeritus Professor Edwin Stanton Good of the Animal Industry Group, University of Kentucky, passed away on April 15, 1957, at the age of 86 years. He received the B.S. degree from Michigan State Agricultural College in 1903, and the M.S. degree from the University of Illinois in 1906, where he also served as Instructor and Investigator in Animal Husbandry. His death terminated a half century of distinguished service at the University of Kentucky. He relinquished his administrative duties in 1941 but he continued his service in Extension and Resident Teaching for several years thereafter.

Perhaps his greatest scientific contribution was the isolation of, and the preparation of a vaccine against, the bacillus causing abortion in mares. He made other contributions in animal nutrition. He was also a good judge of livestock.

The American Society of Animal Production, of which he was a Charter Member, honored him in 1940 when it hung his portrait in the Gallery of the Saddle and Sirloin Club. He was a member of several other professional and learned societies.

His wife preceded him in death some four years ago. Two sons survive.

Henry William Vaughan

Henry William Vaughan, former professor of animal husbandry at the Montana State College and widely and favorably known to the stockmen of Montana, died at the Deaconess Hospital on November 9, the result of an automobile accident near Hardin on August 24.

Vaughan was born February 8, 1887, at Cardington, Ohio, the son of Edgar J. Vaughan and Mary Mooney. He was married to Nelle Page, September 8, 1915, at Charles City, Iowa.

As an animal husbandman Vaughan had a notable career. Graduating from Ohio State University in 1908, he took his master's degree at the Iowa State College in 1913. He was professor of animal husbandry at Iowa from 1913 to 1919, when he accepted a position as professor of animal husbandry at the University of Minnesota. In 1927 he came to Montana State College as head of the animal husbandry department.

Under his leadership the department attained a position in the eyes of Montana stockmen it had never reached at any other time and his students universally testify as to the worth of his teaching.

Vaughan did much original research in animal husbandry and was the author of standard textbooks widely used in most colleges; "Types and Classes of Livestock" published in 1915, with new editions off the press until 1941; "Breeds of Livestock in America", published in 1931, and countless short articles and technical papers. At one time he was editor of the official Duroc Jersey magazine. An excellent judge of livestock, Vaughan's services were widely sought at various livestock shows and expositions.

Vaughan resigned from the college faculty in 1950 and for a number of years has lived on a small ranch south of the Hot Springs, where he devoted his time to producing fine Hampshire sheep.

He was a member of the honorary agricultural fraternity Alpha Zeta and of Sigma Chi, in which fraternity he always took a keen interest.

Vaughan leaves his wife, Nelle Page Vaughan; a son, David P. Vaughan, Bozeman; a daughter, Mrs. Charles (Mary) Wildensten, Berkeley, California; a brother, E. J. Vaughan, Saginaw, Michigan, and five grandchildren.

D. P. Waldorf

Donald Paul Waldorf was born in Chicago, Illinois, November 10, 1932. His death occurred in Madison, Wisconsin, on October 29, 1957. He is survived by his wife, Margaret Ann of Madison, his parents, Mr. and Mrs. Paul E. Waldorf, and his sister, Judy Ann, of Timonium, Maryland, and his grandparents, Mr. and Mrs. H. H. Bray of Redkey, Indiana.

Donald received the Bachelor's degree from Purdue University in 1954. He was the recipient of a Ralston-Purina Fellowship and did graduate work at the University of Wisconsin, receiving the M.S. degree in 1955 and the Ph.D. degree in August of 1957. He had been a member of the American Society of Animal Production for three years. Death came as he was in the terminal stages of preparing his research material for publication and while he was awaiting induction into the Armed Forces.

Alfred Leroy Baker

Alfred Leroy (Fred) Baker, veteran beef cattle research specialist and field station superintendent for the Animal Husbandry Research Division, Agricultural Research Service, United States Department of Agriculture, died of cancer on September 25, 1957 at Colorado Springs, Colorado. He was born at Lakeview, Michigan, January 27, 1898. He left the Montana State College to serve for two years in the United States Marine Corps during World War I, but returned to take his B.S. degree in Animal Husbandry in 1921. He later took some post-graduate work in Animal Genetics at the University of Minnesota. Following graduation he became livestock assistant for the Montana Experiment Station at the Havre Substation, where he served from 1921–23. He entered the service of the Bureau of Animal Industry as animal husbandman on early cooperative work there, transferring to the U. S. Range Livestock Experiment Station at Miles City, Montana, when it was set up in 1924. In the course of his long career with the Department he served as beef cattle research specialist at Miles City and in the Denver office, and as station superintendent at the Ardmore, South Dakota, Jeanerette, La., and Front Royal, Va. beef cattle research stations. He was author or co-author of numerous Montana State College and USDA technical bulletins and Journal of Animal Science and popular articles on livestock subjects, principally beef cattle. His last official station was as beef cattle specialist at the Southern Great Plains Station at Woodward, Oklahoma, from which he retired on disability March 31, 1957, after more than 33 years service with the Department. Mr. Baker was a member of the Sigma Alpha Epsilon, Alpha Zeta, and Masonic fraternities and of the Rotary Club, the American Legion, and the American Society of Animal Production. He is survived by his wife Catherine, a son Dr. Bruce A. Baker of Colorado Springs and two daughters, Janet P. and Barbara G.

NEWS AND NOTES

The European Association for Animal Production will hold its annual meeting in Brussels, Belgium, June 23 to 27. The theme will be: Productivity in farm animals. Visitors from the American Society of Animal Production will be welcomed. For details about the program and the location of the meeting address Mr. K. Kallay, Secretary General, Federation Europeene de Zootechnie, c/o Food and Agriculture Organization, Rome, Italy.

The Western Section of the American Society of Animal Production meetings will be held on July 14, 15 and 16 at the University of California, Davis.

Harry A. Keener, Professor of Dairy Husbandry, University of New Hampshire, has been appointed director of the Agricultural Experiment Station effective January 1, 1958. He has been at the University of New Hampshire since 1941. Dr. Keener will continue on a part time basis as a member of the Dairy Husbandry Department, supervising the research program in dairy cattle nutrition, working with graduate students in that area and doing some instructional work.

Ralph E. Comstock, formerly a staff member of the Institute of Experimental Statistics, North Carolina State College, has joined the University of Minnesota staff as Professor of Animal Husbandry. Dr. Comstock succeeds L. M. Winters, who reitred August, 1956. Dr. Winters is presently serving as a Consultant in the ICA program, stationed at Bagdad, Iraq.

Olaf E. Kolari, has been appointed Assistant Professor of Animal Husbandry at the University of Minnesota, where he will work in beef cattle nutrition.

Donald M. Thrasher recently completed a Ph.D. degree in Animal Nutrition at Purdue University and has joined the Animal Industry Department at Louisiana State University as Assistant Professor in charge of Swine Teaching and Research.

Auttis M. Mullins recently completed a Ph.D. degree at Missouri and has joined the Animal Industry Department at Louisiana State University as Assistant Professor in charge of Meats Teaching and Research.

T. J. Cunha's recent text, "Swine Feeding and Nutrition", which was published by Interscience Publishers, Inc., New York and London, is an outstanding contribution to the animal industry. Dr. Cunha, Head of the Department of Animal Husbandry and Nutrition, University of Florida, has made use of his own research experience and his knowledge of the literature in compiling a text that is complete and up to date in the field of swine nutrition. The book is well illustrated, readable and well organized. It should be useful to the researcher, feed manufacturer, swine grower and others interested in swine production. It is an excellent reference for both historical and recent investigations pertaining to swine nutrition. Teachers of swine management and production courses should find it useful as a guide to good swine feeding practices. Persons formulating swine rations should find it helpful in the technical aspects of complex feed formulation. This is perhaps the only book that contains a complete digest of knowledge in the field of swine nutrition.—R. H. Grummer.

A. J. Dyer was named head of the Animal Husbandry Department at the University of Missouri effective September 1, 1957. He succeeds L. A. Weaver, who retired on that date and was made Professor Emeritus. Dr. Dyer has been a member

of the Animal Husbandry Department since 1938, with the exception of three years of military service during World War II and a year's leave of absence during 1954–55, when he worked with the Kansas City Livestock Exchange and Stockyards Company.

G. R. Johnson, formerly Associate Professor of Animal Science, was appointed head of the Animal Science Department at Ohio State University effective January 1, 1958. In the new position he succeeds Prof. L. A. Kaufman, who retired for health reasons.

R. M. Koch, superintendent of the Beef Cattle Research Station, Fort Robinson, Nebraska, for the past three years, has returned to the staff of the Department of Animal Husbandry at the University of Nebraska.

J. E. Ingalls has been named acting superintendent of the Fort Robinson Station.

C. Jim Elam and Jose Gutierrez have joined the staff of the Beef Cattle Research Branch, Animal Husbandry Research Division, ARS, Beltsville, Maryland.

Frank A. Hudson, who recently completed his Ph.D training at Oregon State College, has been appointed to a position of animal husbandryman at the Sheep, Goat, and Fur Fiber Research Branch of AHRD, ARS, Beltsville, Maryland.

M. A. MacDonald has returned to Canada from New Zealand where he served for two years as Beef Cattle Research Officer at the Ruakura Animal Research Station. Since then he has completed a Post Doctorate Fellowship at the Department of Animal Husbandry, University of Saskatchewan and now has taken up the position of Research Officer (Animal Physiologist) at the Lethbridge Experiment Station, Lethbridge, Alberta.

Dr. R. T. Clark, Coordinator, Beef Cattle Breeding Research, Agricultural Research Service, U. S. Department of Agriculture, Denver, Colorado, left the United States on January 12 to undertake a one-year assignment as Animal Ecologist in Argentina under the Expanded Technical Assistance Program of the Food and Agriculture Organization of the United Nations.

Training Program for Steroid Biochemistry. Applications are now being accepted for the third course in the Training Program for Steroid Biochemistry which will begin October 1, 1958. The program is sponsored by the National Cancer Institute of the National Institutes of Health.

Two groups of candidates will be selected for training. Post-doctoral candidates having an M.D. or a Ph.D. degree will receive $5,000 for a one year training period extending from October 1, 1958 through September 30, 1959. Candidates having a B.S. or M.S. degree or equivalent training will receive $1,500 for a six months' period extending from October 1, 1958 through March 31, 1959.

Closing date for applications will be June 1, 1958. Requests should be made to the Training Program for Steroid Biochemistry, Department of Chemistry, Clark University, Worcester, Massachusetts, or to Dr. Kristen Eik-Nes, Department of Biochemistry, College of Medicine, University of Utah, Salt Lake City, Utah.

Dr. James H. Bywaters, Professor of poultry husbandry, Virginia Polytechnic Institute and in charge of poultry husbandry research at the Virginia Agricultural Experiment Station since 1947, has been named Director of Research, The William H. Miner Agricultural Research Institute, Chazy, New York effective February 1, 1958.

INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, double-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should consist only of a paragraph indicating very briefly the nature of the question asked and the reason for asking it. Reference to relevant literature should be made later in the discussion of the results. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results or their relation to some problem of the livestock industry. Each paper should end with a brief summary in the form of an abstract containing the most pertinent data in the briefest possible form.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. There will be a charge for making engravings in excess of one page per paper and of printing tables in excess of two pages per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual that will assume responsibility for the charges and for the reprints ordered at that time.

6. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts." *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest to members. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. W. D. Gallup, Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma, or to the publishers: Boyd Printing Company, 372–378 Broadway, Albany 7, New York.

12. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, Dr. H. H. Stonaker, Department of Animal Husbandry, Colorado State University, Fort Collins, Colo., or to the publishers, Boyd Printing Company, 372–378 Broadway, Albany 7, New York.